

# Evaluation of Hypoglycemic activity of *Thymus serpyllum* Linn in glucose treated mice

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**Abstract** – *Thymus serpyllum* Linn. was evaluated for its hypoglycemic activity in 10 % d-glucose treated mice. The blood glucose level of glucose-fed mice treated with aqueous extract of *Thymus serpyllum* Linn. for one month remained within the normal range while the blood glucose level of glucose-fed untreated mice was significantly ( $P < 0.001$ ) increased as compared to control. The aqueous extract also exhibited a significant ( $P < 0.05$ ) increase in the weight of pancreas, heart and kidneys of the d-glucose-treated mice. The intraperitoneal (i.p) median lethal dose (LD50) of the aqueous extract of *Thymus serpyllum* was found to be 1050 mg/kg in mice. The result obtained from this study showed that the aqueous extract of *Thymus serpyllum* possesses hypoglycemic activity and supports the ethno medical claim of the use of the plant in treatment of hyperglycemic states.

**Keywords** – *Thymus serpyllum* Linn, hypoglycemic activity, acute toxicity, mice

## 1. Introduction

Diabetes mellitus (DM) is a chronic disorder caused by decreased insulin production in the pancreas, or by the ineffectiveness of the insulin action on target tissues. This result in increased concentrations of glucose in the blood which in turn damage many of the body's systems particularly the blood vessels and nerves [1]. The excess glucose produced in the body reacts with hemoglobin (Hb) to form glycosylated hemoglobin (HbA1c) which is used as marker to determine the degree of protein glycation during diabetes mellitus. The advanced glycated end products (AGEs) are associated with severe diabetic complications [2, 3]. Many of the complications induced by diabetes are suspected to be mediated by oxygen free radical generation [4]. The history of diabetes dates back to the ancient times and has remained a significant threat to life till date [5]. Diabetes affected more than 300 million people worldwide [6]. Oral anti-hyperglycemic agents and insulin are often used for management of diabetes but they have prominent side effects and fail to significantly alter the course of diabetic complications [7]. In addition, they are not suitable for use during pregnancy [8]. Since ancient times, diabetics have been treated with several medicinal plants or their extracts based on folklore medicine [9]. The drugs obtained from plants are gaining popularity both in developing and developed countries because of their natural origin and less side effects [10]. World Health Organization has listed 21,000 plants which are used for medicinal purposes around the world [11]. According to WHO, the use of medicinal plants to treat diabetes mellitus needs further investigation [12]. Therefore, the search for more effective and safer hypoglycemic agents has continued to be an important area of active research. *Thymus serpyllum* (wild

thyme) is locally known as Tumuro and belongs to the family Labiatae. *Thymus serpyllum* has been extensively used in the folk medicine for many years [13]. *Thymus serpyllum* has been reported to exhibit antioxidant activity [14] and antioxidants have been demonstrated to attenuate the oxidative damage that leads to severe diabetic complications. Antioxidants have been considered as treatment of diabetes [15]. It is reported that *thymus serpyllum* possess antioxidant activity. The present study was carried out to evaluate the hypoglycemic effect of *Thymus serpyllum* L. in mice.

## 2. Materials and Methods

### 2.1. Equipments

Optium xceed glucometer (Abbott Laboratories, USA), rotary evaporator, electrical weighing balance, dissection box and desiccator.

### 2.2. Chemicals

D-glucose, chloroform and normal saline. Chemicals were purchased from Sigma Chemicals Co.

### 2.3. Collection, identification and preparation of plant Extract

The selected plant was collected from the mountains of the village Shikiyote; district Gilgit, Gigit-Baltistan, Pakistan. It was identified and authenticated by Dr. Shair Wali, Assistant professor of Botany, Karakoram International University, Gilgit-Baltistan. Plant material was shade dried and powdered with a Chinese herbal grinder. The powdered material was stored in well closed cellophane bags at 4 °C in the refrigerator. The powdered plant was extracted by method of cold maceration. The powder was soaked in distilled water for 48 hours with occasional shaking. It was passed through

muslin cloth and then filtered through the filter paper. The extract was dried with the help of rotary evaporator.

2.4. *Animals*

Adult healthy mice (25-30g) of either sex were used in the study. All animals were housed at the animal house of faculty of Pharmacy University of Sargodha. Animals were housed under standard laboratory condition (light period 8.00 a.m to 8.00 p.m, 23±2 °C , relative humidity 55%), standard pellet diet and water *ad libitum*. Animals received human care in accordance with NIH guidelines and the study protocol was approved by the local ethical committee Faculty of Pharmacy University of Sargodha.

2.5. *Collection of blood for biochemical analysis*

The mice were anesthetized by using chloroform and blood samples were collected by direct cardiac puncture. Blood glucose level was measured by Optium Exceed Glucometer using glucose oxidized optimum kits (Abbott Laboratories, USA).

2.6. *Hypoglycemic effect of aqueous extract of Thymus serpyllum in glucose-fed mice*

The chronic administration of d- glucose in drinking water results in hyperglycemia [16]. The aim of this study was to evaluate the hypoglycemic effect of aqueous extract of *Thymus serpyllum* in glucose-fed mice for one month. Mice were divided into three groups of ten mice each. Group-1 served as the normal control and was provided only with distilled water *ad libitum*. Group-2 served as glucose-fed untreated group and was provided with 10 % d-glucose in drinking water for one month. Group-3, glucose-fed treated group was given 10% d-glucose in drinking water and administered orally 500 mg/kg body weight of aqueous extract of *Thymus serpyllum* daily for one month. At the end of the month, glucose levels of all mice in three groups were estimated.

2.7. *Effect of aqueous extract of Thymus serpyllum on body weight, heart, kidneys, liver and pancreas of in glucose-fed mice*

This experiment was performed to check the effect of aqueous extract of *Thymus serpyllum* on body weight and weight of the heart, kidneys, liver and pancreas of mice chronically fed with d-glucose for one month. Body weight of mice was measured at the end of the month using electrical weighing balance. Then, the mice were sacrificed under anesthesia and the weights of pancreas, liver, heart and kidneys of all the mice in three groups were measured.

2.8. *Acute toxicity studies in mice*

The aim of this experiment was to determine the LD<sub>50</sub> of the crude extract. Adult healthy mice were randomly divided into five groups of 2 animals each. Group I served as control and received normal saline 10ml/kg. Group 2, 3, 4 and 5 were given different doses of aqueous extract of *Thymus serpyllum* in an ascending order i.e 100, 300, 600, 900 mg/kg respectively. The animals were observed continuously for 3 h and then they were observed each hour during 24 h after administering the extract to observe any changes in general behavior or other physiological activities. The mortality rate was observed for 24 hours. Since no mortality occurred so, another five groups of mice were taken. They were again

treated with the various doses of aqueous extract in an increasing order i.e 1200, 1500, 1800, 2000 mg/kg respectively. All the doses were administered intraperitoneally. The highest dose, which did not kill any animals, and the lowest dose, which killed only one mouse, was noted. LD<sub>50</sub> was calculated from the geographic mean of these two doses [17].

2.9. *Statistical analysis*

Values were represented as Mean ± SEM and data were analyzed by ANOVA followed by Turkey’s test. A value of P<0.05 was considered significant.

3. **Results**

3.1. *Hypoglycemic effect of aqueous extract of Thymus serpyllum in glucose-fed mice*

The blood glucose level of glucose-fed mice treated with aqueous extract did not significantly change as compared to normal control group while, the blood glucose level of untreated glucose-fed mice was highly significantly increased (P<0.001) as compared to normal control. (Table 1 and Figure 1 ).

Table 1. Blood glucose level of normal control, glucose-fed and glucose-fed treated mice at the end of one month

Grouping of mice	Mean blood glucose level (mg/dl)
Normal control (water <i>ad libitum</i> )	94.60±7.05
Glucose-fed (10 % d-glucose in drinking water)	204.70±7.05 <sup>c</sup>
Glucose-fed treated (10 % d-glucose in drinking water) with (500 mg/kg (b.w) of extract	9.04±7.05 <sup>ns</sup>

Data is expressed as Mean ± SEM (n=10), where c = P<0.001 compare to control and ns= non-significant compared to normal control

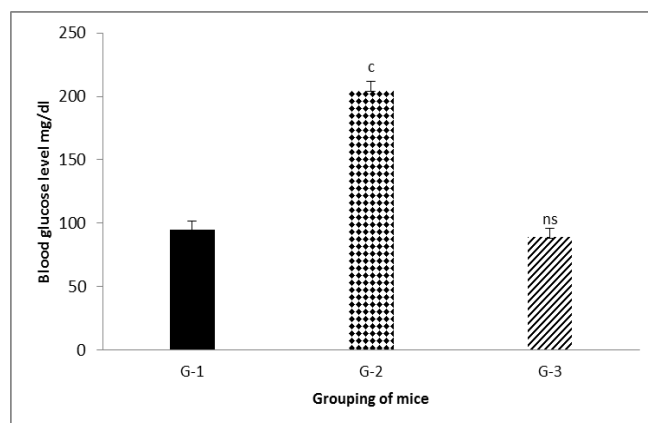


Figure 1. Blood glucose level of normal control, glucose-fed and glucose-fed treated mice where c = P<0.001 and ns = non-significant compared to normal control. G-1 = Normal control, G-2 = glucose-fed, G-3 = glucose-fed treated mice.

### 3.2. Effect of aqueous extract of *Thymus serpyllum* on body weight, heart, kidneys, liver and pancreas of glucose-fed mice

The weight of pancreas, heart and kidneys of the glucose-fed treated mice was significantly ( $P < 0.05$ ) increased as

compared to the glucose-fed mice. The whole body weight of mice in all three groups was not significantly changed (Table 2).  $LD_{50}$  of the aqueous extract of *Thymus serpyllum* was found to be 1050 mg/kg.

Table 2. Body weight, heart, kidneys, liver and pancreas of normal control, glucose-fed and glucose-fed treated mice at the end of one month.

Group of Mice	Body weight of Mice (gm)	Heart weight (gm)	Kidneys weight (gm)	Liver weight (gm)	Pancreas weight (gm)
Normal control	28.4±0.84	0.244±0.12	0.474±0.27	1.53±0.10	0.195±0.01
Glucose-fed	27.1±0.84	0.188±0.12	0.440±0.27	1.41±0.10	0.178±0.01
Glucose-fed treated	28.5±0.84	0.241±0.12 <sup>a</sup>	0.602±0.27 <sup>a</sup>	1.78±0.10 <sup>a</sup>	0.205±0.01 <sup>a</sup>

Data is expressed as Mean ± SEM (n=10) significant at  $P < 0.05$ , vs glucose-fed mice, where a =  $P < 0.05$ .

## 4. Discussion

Plants have been used from ancient times to cure diseases of man and animals. Despite the progresses in modern medicine, it has been reported that more than 70% of the developing world's population still depends on the traditional medicine [18]. The ethnobotanical studies have revealed that about 800 plants may possess anti-hyperglycemic properties [19]. Present study was conducted to investigate the hypoglycemic activity of aqueous extract of *Thymus serpyllum* Linn. in mice chronically fed with d-glucose for one month. The effect of aqueous extract on the body weight and weight of the heart, kidneys, liver and pancreas of glucose-fed mice was also checked. It was further aimed to determine median lethal dose ( $LD_{50}$ ) of the aqueous extract of *Thymus serpyllum*. The blood glucose level of glucose-fed mice treated with aqueous extract for one month remained within the normal range while the blood glucose level of glucose-fed untreated mice was significantly ( $P < 0.001$ ) increased as compared to control. Previously it was shown that chronic glucose feeding for 3 weeks resulted in non-insulin-dependent diabetes as reflected by an increase in blood glucose level [16]. The most important outcome of diabetes is the high blood glucose concentrations (hyperglycemia). Chronic exposure to high glucose concentration can lead to cellular dysfunction that may become irreversible over time, a process that is termed glucose toxicity. It has been reported that the nonphysiological and potentially irreversible  $\beta$ -cell damage is caused by the supraphysiological glucose concentrations for a long time [20]. The aqueous extract of *Thymus serpyllum* inhibited the blood glucose level to increase in mice exposed to the chronic high glucose concentrations. In the present study prevention of blood glucose elevation in mice fed with chronic glucose might be due to the increased clearance of glucose by the aqueous extract. Hyperglycemia is clinical hallmark of diabetes mellitus and leads to many adverse effects on the vital organs of the body including the  $\beta$ -cells damage. Oxidative stress has also been considered as an explanation for the tissue damage that accompanies

chronic hyperglycemia [21]. *Thymus serpyllum* has been reported to exhibit antioxidant activity and antioxidants have been considered as treatment of diabetes [14, 15]. The chronic high blood glucose level produces stress and leads to the formation of oxygen reactive species. It is suggested that the phytochemical constituents of the aqueous extract demonstrated a protective effect on the  $\beta$ -cells against the oxidative damage of high glucose concentration. Previous studies have shown that the certain phytochemical compounds e.g flavonoids of antioxidant plants play a protective role against the reactive oxygen species (ROS) that have cytotoxic effect on vital organ of the body, in particular the pancreas [20]. Midoui and Champlain (2002) suggested the involvement of oxidative stress in the development of insulin resistance. Recent studies have shown that antioxidant  $\alpha$ -Lipoic Acid improves insulin sensitivity in patients with type 2 diabetes [22]. It has also been studied that antioxidants improve the effects of insulin on skeletal muscle glucose transport in animal models of insulin resistance [23]. Moreover, it has been demonstrated that treatment with antioxidants improves impaired insulin-mediated glucose uptake in high glucose-fed rabbits [24]. From these previous studies it is suggested that the hypoglycemic activity of aqueous extract in glucose-fed mice may also be associated to the improvement of insulin resistance against oxidative stress of chronic glucose administration. Glycation of proteins associated with the generation of free radicals is believed to be underlying factor in certain pathologies of diabetes [25]. Although several mechanisms have been proposed for the pathogenesis of chronic diabetic complications, protein glycation and oxidation by glucose (glycooxidation) are considered the important mechanisms [26]. Interestingly, previous studies have demonstrated that decreasing the oxidative stress, would be effective in preventing protein glycation thus reducing the development of diabetic complications [16]. Hence, considering above mentioned assumption it may be suggested that the aqueous extract of *Thymus serpyllum* by the virtue of its antioxidant potential may have beneficial effects in reducing the diabetic complications.

Acute fluid loss during diabetes leads to the weight loss [27]. The aqueous extract prevented the weight loss in glucose-fed treated mice during one month study while, the weight loss occurred in the glucose-fed mice, however the result was non-significant. The result agrees with the previous study conducted [28]. It has also been studied that *Azadirachta indica* (Neem), *Allium sativum* (Garlic) extracts improved the body weight gain in Streptozotocin induced diabetic rats [28]. Aqueous extract of *Thymus serpyllum* significantly ( $P < 0.05$ ) increased the weight of pancreas, heart and kidneys of the glucose-fed treated mice as compared to the glucose-fed mice. In a previous study, the normal rats treated with extract of *Elaeocarpus grandiflorus* increased weights of heart, kidney and pancreas. It has been shown previously that plant extract significantly reduced the diabetic effect on the kidney and liver weights [29]. It is suggested that the aqueous extract brought about significant beneficial effects against the cytotoxic effects of reactive oxygen species on these vital organs and leads to the improvement of their weights. The intraperitoneal (i.p) median lethal dose ( $LD_{50}$ ) of the aqueous extract of *Thymus serpyllum* was found to be 1050 mg/kg in mice. The administration of the aqueous extract from 100 to 900 mg/kg body weight did not produce significant changes in behavior, breathing, sensory and nervous system responses in mice. During 24 h of the monitoring, no deaths occurred in any of the groups. These results showed that the aqueous extract up to the dose of 900 mg/kg body weight in mice is safe as it produced no adverse effects.

## 5. Conclusion

It is concluded that the hypoglycemic activity of *Thymus serpyllum* Linn. might be due to the increased clearance of glucose and the antioxidant effect of the aqueous extract. However, further studies are needed to isolate the active hypoglycemic principal (s) and explore its exact mechanism of action.

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