Comparative hypoglycemic activity of different fractions of *Thymus serpyllum* L. in alloxan induced diabetic rabbits

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Abstract: The aim of present study was to evaluate and compare the hypoglycemic activity of different solvents extracts of *Thymus serpyllum* in rabbits. Diabetes was induced with single intravenous injection of alloxan monohydrate (150mg/kg). Glibenclamide and acarbose were used as standard drugs. The crude powder of *Thymus serpyllum* (500 mg/kg b.w) significantly reduced blood glucose level in both normal and diabetic rabbits. Various extracts of *Thymus serpyllum* were compared for their hypoglycemic activity in diabetic rabbits. Ether and aqueous extracts significantly reduced the blood glucose level with maximum effect ($p<0.001$) produced by aqueous extract, which was selected for further study. Aqueous extract significantly inhibited the rise in glucose level in oral glucose tolerance test. The extract showed synergistic effect with different doses of insulin; however serum insulin level of the diabetic rabbits was not significantly increased by the extract. HbA1c level was significantly ($p<0.05$) reduced whereas hemoglobin level was significantly increased in three months study. Phytochemical screening of the aqueous extract showed the presence of alkaloids, flavonoids, tannins, terpinoids, reducing sugar and cardiac glycosides. It is concluded that the aqueous extract might be used alone or in combination with insulin to manage diabetes and its associated complications.

Keywords: Aqueous extract, blood glucose level, insulin, *Thymus serpyllum* L.

INTRODUCTION

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs. These synthetic drugs are expensive and have a number of serious adverse effects. Medicinal plants have been used for the treatment of different diseases including diabetes for a long time. In the past decade, research has been focused on scientific evaluation of local plant derived drugs and screening of more effective and safe hypoglycemic agents. *Thymus serpyllum* (wild thyme) belongs to the family Labiatae and locally known as Tumuro. In Pakistan; it is found in Gilgit-Baltistan, Kashmir and Jamu. *Thymus serpyllum* has been traditionally used in numerous diseases. It is used for ailments of the respiratory, digestive and genito-urinary system. Its leaves are laxative and good tonic for kidney and eye diseases. It is effective in bronchitis and for purification of blood. The oil is remedy for toothache. It is given in weak vision, complaints of liver, suppression of urine and menstruation (Qureshi, 2007). *Thymus serpyllum* has the anti-diabetic potential because of its α-glucosidase inhibiting activity (Gholamhoseinian *et al*., 2008). Previously we have investigated the hypoglycemic and hypolipidemic effect of crude extract of *Thymus serpyllum* Linn. in normal and alloxan- induced diabetic rabbits (Alamgeer *et al*., 2012). The current study was conducted to compare the hypoglycemic activity of various extracts of *Thymus serpyllum* and to study in detail effect of the most potent extract on insulin and HbA1c level.

MATERIALS AND METHODS

Chemicals and drugs

Alloxan monohydrate, ether, ethanol and chloroform were purchased from Sigma Chemicals Co. Glibenclamide and acarbose were obtained from Biorax Pharmaceuticals, Islamabad and Baeyer's pharmaceuticals, Karachi, Pakistan respectively.

Animals

Adult healthy rabbits (1.0-1.5kg) 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%), green fodder and water *ad libitum*. Animals received human care in accordance with NIH guidelines and the study protocol was approved by the local ethical committee.

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Plant material and extraction

The selected plant was collected from the mountains of the village Shikyote; district Gilgit-Baltistan, Pakistan. It was identified and authenticated by Dr. Shair Wali, assistant professor of botany, Karakoram International University, Pakistan. A voucher specimen was preserved at faculty of Pharmacy University of Sargodha, Pakistan, with voucher (No.TS-20) for future reference. Plant material was shade dried and powered with a Chinese herbal grinder. The powdered material was stored in well closed cellophane bags at 4ºC in the refrigerator. The powder was successively extracted by method of cold maceration using solvents; ether, chloroform, ethanol and distilled water and percentage yields of extracts were 2.9%, 2.4%, 3.1% and 4.2% respectively.

Hypoglycemic activity of crude powder in normal and diabetic rabbits

After an overnight fasting, rabbits were divided into five groups of six animals each. Group-1 served as untreated normal control and was administered orally 20 ml of 2% gum tragacanth solution in water only. Group-2 and 3 were given orally 250 and 500mg/kg body weight of crude powder of *Thymus serpyllum* respectively. Group-4 and 5 were given orally 3mg/kg body weight of glibenclamide and acarbose respectively (Andrade-Cetto and Heinrich, 2005). Blood glucose levels were measured by Optium Xceed Glucometer using glucose oxidized optium kits (Abbott Laboratories, USA) at 0, 2, 4 and 6 hours intervals after drugs administration.

The same procedure of grouping was adopted for diabetic rabbits by adding another group of diabetic control animals. Diabetes was induced with a single intravenous injection of 150mg/kg body weight of alloxan monohydrate dissolved in isotonic saline. After 72 h of alloxan administration, induction of diabetes was confirmed by measuring the blood glucose level. Rabbits with blood glucose level between 250 to 300 mg/dl were considered diabetic and studies were carried out on these diabetic rabbits (Akhtar *et al*., 2002).

Screening of hypoglycemic activity of different extracts

Diabetic rabbits were divided into five groups of six animals each. Group-1 served as untreated diabetic control and was administered orally 20 ml of 2% aqueous gum tragacanth solution. Group-2, 3, 4 and 5 were administered ether, chloroform, ethanolic and aqueous extracts of *Thymus serpyllum* 500 mg/kg (b.w) of each extract respectively. The blood glucose levels were checked at 0, 2, 4 and 6 hours intervals after the administration of drugs.

Oral glucose tolerance test (OGTT)

Rabbits were divided into four groups of six rabbits each. Group-1 served as untreated normal control and was administered orally 2 ml/kg (b.w) of distilled water. Group-2 received 500 mg/kg body weight of aqueous extract. Group-3 and 4 received orally 0.01mg/kg body weight of glibenclamide and 3mg/kg body weight of acarbose respectively. 30 minutes after the drugs administration, all the rabbits were given oral glucose load at 1g/kg body weight (Perfumi *et al*., 1991). Blood glucose levels were measured for each group of animals at 0, 0.5, 1, 2, 4 and 6 hours of administering the drugs.

Comparison of hypoglycemic activity of aqueous extract with or without insulin in diabetic rabbits

Diabetic rabbits were divided into five groups of six animals each. Group-1 was administered subcutaneously 6 units/kg body weight of insulin. Group-2, 3 and 4 were administered subcutaneously 3, 2 and 1 units/kg body weight of insulin plus 500 mg/kg body weight of aqueous extract respectively. Group-5 was administered orally aqueous extract of *Thymus serpyllum* in a dose 500 mg/kg (b.w) only. Blood glucose levels were estimated at 0, 0.5, 1 2, 3, 4 and 6 hours interval after the administration of insulin and aqueous extract (Ahmad *et al*., 2009).

Effect of aqueous extract on serum insulin level of diabetic rabbits

Rabbits were divided into three groups of six animals each. Groups-1 and 2 served as untreated normal and diabetic control and were administered orally 20ml of 2% aqueous gum tragacanth solution daily for one month. Group-3 was administered orally 500mg/kg body weight of aqueous extract daily for one month. At the end of the month, the blood samples of all rabbits in each group were collected for the estimation of serum insulin level by ELIZA reader (Stat Fax 2100, Awareness Technology, Inc USA).

Effect of aqueous extract of on hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) levels in diabetic rabbits

Rabbits were divided into five groups of six animals each. Groups-1 and 2 served as untreated normal and diabetic control and were administered orally 20 ml of 2% aqueous gum tragacanth solution daily for 3 months. Group-3 and 4 received orally 600µg/kg body weight of glibenclamide and 3 mg/kg body weight of acarbose respectively daily for 3 months. Group-5 was administered orally 500 mg/kg body weight of aqueous extract of *Thymus serpyllum* daily for 3 months. At the end of the 3 months, the blood samples of all rabbits in each group were collected in glass tubes treated with EDTA K_3 to estimate the Hb and HbA1c levels (Home *et al*., 2008; Knowler *et al*., 2002).

Phytochemical analysis

Phytochemical analysis of aqueous extract of *Thymus serpyllum* was performed for the presence of alkaloids, indole alkaloids, saponis, flavonoids, tannins, reducing sugars, steroids, glycoside, anthraquinone glycosides and terpenoids (Inwukaeme *et al*, 2007).

Table 1: Blood glucose level of normal rabbits treated with 2 % gum tragacanth solution, crude powder of *Thymus serpyllum,* glibenclamide and acarbose.

Time (hours)	Normal control 2% gum tragacanth MBGL (mg/dl)	Crud powder of *Thymus sepyllum* (250 mg/kg) MBGL (mg/dl)	Crude powder of *Thymus sepyllum* (500 mg/kg) MBGL (mg/dl)	Glibenclamide (3 mg/kg) MBGL (mg/dl)	Acarbose (3 mg/kg) MBGL (mg/dl)
0	109.5±4.78	112.3±4.30	123.1±4.68	107.1±4.98	110.1±4.76
1	108.3±4.38 ^{a}	104.83±4.3 ^{a}	97.8±5.82 ^{b}	90.1±5.02 ^{b}	92±5.81 ^{b}
2	106.1±4.45 ^{a}	97.1±3.85 ^{a}	82±5.2 ^{c}	85.8±4.26 ^{c}	84.5±3.53 ^{b}
4	104.3±4.72 ^{a}	100.6±5.30 ^{a}	73.66±4.03 ^{c}	79.1±3.05 ^{c}	78.5±1.56 ^{b}
6	104.8±5.095 ^{a}	109.3±4.44 ^{a}	82.3±6.10 ^{b}	73.6±2.61 ^{c}	78.1±2.27 ^{b}
Mean	106.6±1.98 ^{A}	104.8±2.12 ^{A}	91.8±3.91 ^{B}	87.2±2.72 ^{B}	88.6±2.73 ^{B}

Data are expressed as Mean ± SEM significant at p<0.05, vs control where (^{a}) = p<0.05, (^{b}) = p<0.01, (^{c}) = p<0.001 and (^{d}) = p<0.0001, the values sharing the same superscript letters in the same column are not significant to each other.

Table 2: Blood glucose level of diabetic rabbits treated with 2% gum tragacanth solution, crude powder of *Thymus serpyllum,* glibenclamide and acarbose.

Time hours	Normal Control 2 % gum tragacanth MBGL (mg/dl)	Diabetic control 2% gum tragacanth MBGL (mg/dl)	Crude powder of *Thymus sepyllum* (250 mg/kg) MBGL (mg/dl)	Crude powder of *Thymus sepyllum* (500 mg/kg) MBGL (mg/dl)	Glibenclamide (3 mg/kg) MBGL (mg/dl)	Acarbose (3 mg/kg) MBGL (mg/dl)
0	109±4.92	265.1±8.0	283.1±6.96	277.8±6.27	265±6.03	273±4.50
1	108.8±4.26^{a}	264.8±6.57^{a}	266.1±7^{b}	232.1±8.44^{b}	249.1±6.40^{b}	254.1±7.4^{b}
2	105.8±4.60^{a}	263.1±7.12^{a}	263.0±8.8^{c}	210.5±12.3^{c}	239±6.34^{c}	237.3±9.4^{c}
4	104.5±5.16^{a}	262.3±7.19^{a}	261.3±4.4^{c}	186.1±5.16^{d}	208.1±6.31^{d}	209.6±5.8^{d}
6	105.3±5.10^{a}	261.6±6.86^{a}	272.6±5.4^{b}	182.8±7.44^{d}	189.6±4.65^{e}	192± 6.20^{e}
Mean	106.7±2.03^{C}	263.4±2.98^{A}	269.2±3.2^{A}	217.9±7.34^{B}	230.20±5.67^{B}	233.2±6.14^{B}

Data are expressed as Mean ± SEM significant at p<0.05, vs control where (^{a}) = p<0.05, (^{b}) =p<0.01, (^{c}) =p<0.001, (^{d}) =p< 0.0001, and (^{e}) = p<0.00001, the values sharing the same superscript letters in the same column are not significant to each other.

STATISTICAL ANALYSIS

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

RESULTS

Hypoglycemic activity of crude powder in normal and diabetic rabbits

Crude powder of *Thymus serpyllum* in a dose of 250 mg/kg did not significantly reduce the blood glucose level while the crude powder in a dose of 500 mg/kg significantly reduced (P<0.05) blood glucose levels of normal and diabetic treated rabbits. The standard drugs, glibenclamide and acarbose also significantly reduced (p<0.05) blood glucose levels of normal rabbits. There was no significant change in blood glucose levels of group-1 receiving 2% aqueous gum tragacanth solution (tables 1 & 2).

Screening of hypoglycemic activity of different extracts

Aqueous extract of *Thymus serpyllum* significantly (p<0.001) reduced the blood glucose level of the diabetic rabbits. Ether extract also produced significant (P<0.01) decrease in the blood glucose level of the diabetic rabbits. There was no significant change in blood glucose levels of the rabbits receiving chloroform, ethanolic and 2% aqueous gum tragacanth solution (fig. 1).

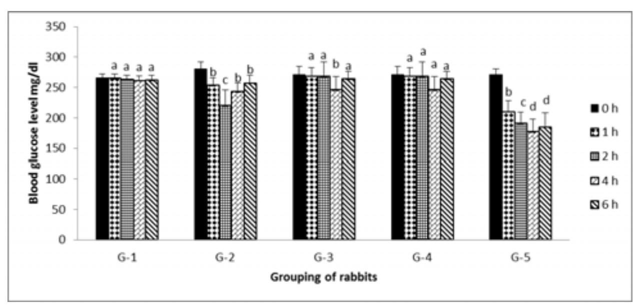

Fig. 1: Blood glucose level of diabetic rabbits treated with various extracts where as a=0.05, b=0.01, c=0.001 and d= 0.0001, the means sharing the same letters are not significant to each other, G-1=Diabetic control, G-2= Ether extract treated, G-3=Chloroform extract treated, G-4=Ethanolic extract treated, G-5=Aqueous extract treated

Oral glucose tolerance test (OGTT) of aqueous extract in normal rabbits

Aqueous extract of *Thymus serpyllum* significantly (p<0.001) inhibited the increase in the blood glucose level after oral glucose load in normal rabbits. The glibenclamide and acarbose also significantly inhibited the increase in blood glucose level in normal rabbits after oral glucose load. Blood glucose level was significantly

(P<0.05) increased in rabbits receiving only 2% aqueous gum tragacanth solution after oral glucose load (fig. 2).

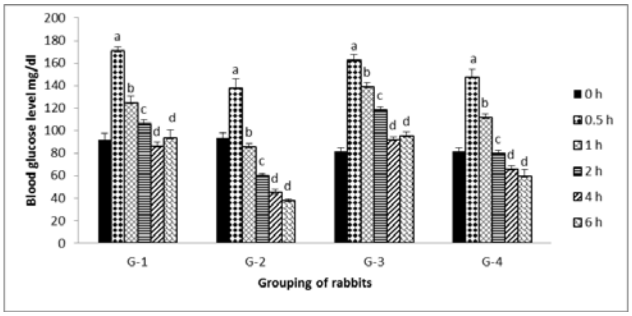

Fig. 2: Blood glucose level of rabbits treated with aqueous extract, Glibenclamide and Acarbose after glucose after oral glucose load where as a=0.05, b=0.01, c=0.001, d=0.0001, G-1=Normal control, G-2=Glibenclamide treated, G-3=Acarbose treated, G-4=Aqueous extract treated.

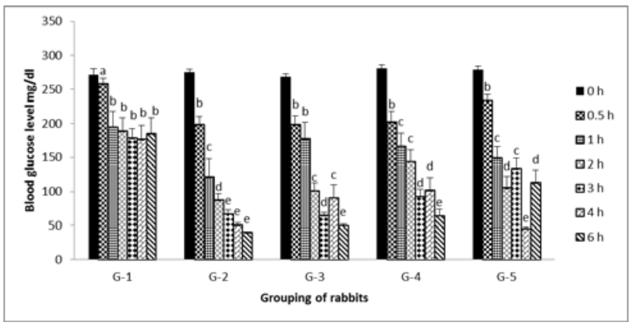

Fig. 3: Blood glucose level of diabetic rabbits treated with *Thymus serpyllum* with and without different doses of insulin where a=P<0.05, b=P<0.01, c=P<0.001, d= P<0.0001 and e=P<0.00001 compared to 0 hour, G-1=aqueous extract (500mg/kg), G-2=Insulin 6 units/kg, G-3=aqueous extract + insulin (3units/kg), G-4= aqueous extract + insulin (2units/kg), G-5=aqueous extract + insulin (1 unit/kg)

Table 3: Serum insulin level of normal control, diabetic control and aqueous extract treated diabetic rabbits after 30 days of treatment.

Grouping of rabbits	Insulin level (IU/dl)
Normal control	3.30±0.26
Diabetic control	1.11±0.26 [a]
Aqueous Extract treated	1.79±0.26 [a]

Data are expressed as Mean ± SEM significant at p<0.05, vs control, the values sharing the same superscript letters in the same column are not significant to each other

Comparison of hypoglycemic activity of aqueous extract of with or without insulin in diabetic rabbits

The aqueous extract of *Thymus serpyllum* produced significant synergistic effect with all doses of insulin used. Synergistic effect of aqueous extract of *Thymus serpyllum* with 3 units/kg of insulin was highly significant (p<0.01) and was comparable to the effect produced by 6 units/kg of insulin alone (fig. 3).

Effect of aqueous extract on serum insulin level of diabetic rabbits

The serum insulin level of the diabetic treated rabbits was not significantly increased by the aqueous extract. However, the insulin level was higher in treated rabbits as compared to diabetic control group (table 3).

Effect of aqueous extract of on hemoglobin (Hb) and Glycosylated hemoglobin (HbA1c) levels of diabetic rabbits

Aqueous extract of *Thymus serpyllum*, glibenclamide and acarbose significantly (<0.05) increased the Hb level and significantly decreased the HbA1c level in diabetic treated rabbits for three months. In diabetic control group, there was a significant decrease in Hb level while HbA1c level was significantly (p<0.05) increased. There was no significant change in Hb and HbA1c level in rabbits of normal (table 4).

Phytochemical analysis

Alkaloids, indole alkaloids, flavonoids, tannins, terpenoids, reducing sugars, steroids and cardiac glycosides were found to be present whereas saponins and anthraquinone glycosides were undetectable in the aqueous extract of *Thymus serpyllum*.

DISCUSSION

A considerable number of hypoglycemic plants and herbs are known through folklore. Ethnobotanical information has indicated that more than 800 plants have been traditionally used for the treatment of diabetes (Alarcon-Aguilar *et al*., 1998) but their introduction into modern therapy awaits pharmacological testing by modern scientific methods. In the present investigation crude powder of *Thymus serpyllum* (500 mg/kg body weight) significantly reduced the blood glucose level both in normal and diabetic rabbits. The results obtained were in accordance with previous studies. Earlier, hypoglycemic response of medicinal plants has been reported in normal and diabetic animals (Ahmad *et al.*, 2009). Hypoglycemic effects of different extracts: ether, chloroform, ethanol and aqueous of *Thymus serpyllum* were compared in diabetic rabbits. The aqueous and ether extracts significantly decreased the blood glucose level with maximum (p<0.001) decrease with aqueous extract up to the period of 6 hours which showed that the compound (s) responsible for hypoglycemic effect is/are extractable more in the water than other solvents used for extraction. Previous studies also demonstrated that aqueous extract of medicinal plants contains certain hypoglycemic agents, which are responsible for its hypoglycemic activity (Ahmad *et al.*, 2009). Chloroform and ether extracts were unable to produce any significant change in the blood

Table 4: Hemoglobin (Hb) and Glycosylated hemoglobin (HbA1c) levels of normal control, diabetic control, glibenclamide, acarbose and aqueous extract treated diabetic rabbits after three months of treatment**.**

Grouping of rabbits	Hemoglobin (Hb) Level (g/dl)	Glycosylated Hemoglobin (HbA1c)%
Normal control	14.55±3.44	3.84±0.87
Diabetic control	8.76±3.44[b]	10.23±0.87[a]
Glibemclamide treated	14.31±3.44[a]	4.69±0.87[b]
Acarbose treated	13.71±3.44[a]	4.19±0.87[b]
Aqueous treated	16.6±3.446[a]	4.33±0.87[b]

Data are expressed as Mean ± SEM significant at p<0.05, vs control where ([a]) = p<0.05, ([b]) = p<0.01, the values sharing the same superscript letters in the same column are not significant to each other.

glucose level of diabetic rabbits, which reflects that constituents in the plant with hypoglycemic activity are not extracted in these solvents. The phytochemical analysis confirmed the presence of alkaloids, indole alkaloids, flavonoids, tannins, terpenoids, reducing sugars, steroids and cardiac glycosides in the aqueous extract. It has been formerly reported that the flavonoids from different plant origin demonstrated promising hypoglycemic activity in animal models. The alkaloids and terpenoids have also been reported to possess significant anti-diabetic potential (Loew and Kaszkin, 2002). The flavonoids are known to possess anti-diabetic activity and have been studied to regenerate the damaged β-cells in alloxan-diabetic rats (Charkravarthy *et al.,* 1980). Glycosides, alkaloid and terpenoids are frequently implicated as having anti-diabetic effect (Malviya *et al*., 2010). Hence, the hypoglycaemic activity of aqueous extract might be due to the presence of these phytochemicals. The inhibition of the intestinal α-glucosidase by the aqueous extract might be involved in lowering the glucose level. Previously *Thymus serpyllum* demonstrated 50% inhibitory effect on α-glucosidase enzyme in an in-vitro investigation (Gholamhoseinian *et al*., 2008).

Subcutaneous administration of insulin in a dose of 6 units/kg b.w significantly reduced the blood glucose level within 2 hours in diabetic rabbits. Insulin (2 units/kg b.w), when co-administered with aqueous extract in a dose of 500 mg/kg given orally reduced significantly blood glucose level in diabetic rabbits. The results were comparable with 6 units/kg b.w of insulin alone. Synergistic effect of aqueous extract with different doses of insulin clearly showed that there might be some biological active principle (s) in aqueous extract of *Thymus serpyllum* that may possess insulin like action. The results obtained agree with the previous study conducted by Maqsood *et al* (2009). It can also be concluded that the aqueous extract may contain some active compound (s), which provide protection against the degradation of the insulin and contribute to its hypoglycemic activity. Hence, the use of aqueous extract in combination with insulin might reduce the units of insulin to produce the reproducible results. So, the adverse effects associated with insulin therapy could be minimized if it would co-administer with the aqueous extract. Several plant species have been found in various studies to be beneficial when used as adjunct therapy in NIDDM (Amala, 2006), which strengthens this idea of combination of insulin with plant extract. Although, the serum insulin level was not significantly increased in diabetic treated rabbits however, the insulin level was higher in treated group as compared to diabetic control animals. So, it is can also be suggested that the aqueous extract has insulin stimulating effect. Previously several plant species has been reported to enhance the insulin release from β-cells in experimental induced diabetes (Patel *et al*., 2012). Oral glucose tolerance test has revealed that the aqueous extract has inhibited the blood glucose level to rise in rabbits after an oral glucose load. It has also been previously reported that administration of leaf extract of *Sphenocentrum jollyanum* in rabbits significantly lowered blood glucose levels after glucose load (Mbaka *et al*., 2008). The aqueous extract significantly increased Hb level while decreasing the HbA1c level. During diabetes, the excess glucose reacts with hemoglobin (Hb) to form glycosylated hemoglobin (HbA1c), which is used as marker to determine the degree of protein glycation. Previously it was reported that *Citrullus colocynthis* fruits extract reduced the HbA1c level thereby by increasing the Hb level in diabetic animals (Jayaraman *et al*., 2009). HbA1c indicates indirect measurement of advanced glycated end products (AGEs which are associated with severe complications of diabetes (Sell *et al*., 1992). So, aqueous extract of *Thymus serpyllum* may be beneficial to reduce the diabetic complications. Oxidative stress has been associated a key factor in the pathogenesis of diabetes. Alloxan acts as a cytotoxic agent for β-cells of pancreas and produces the pancreatic damage by generating the free radical species (Szkudelski, 2001). Diabetes itself increases the production of tissue damaging reactive oxygen species by glucose autoxidation and/or non-enzymatic protein glycation (Signorini *et al*., 2002). *Thymus serpyllum* demonstrated antioxidant activity in a previous study (Pandey *et al*., 1997). So, the active principles in the aqueous extract might protect the β-cells of the islets of Langerhans from oxidative stress and might contribute to the hypoglycemic activity of the aqueous extract.

It is concluded that aqueous extract may be used in combination with insulin for the management of diabetes and associated complications. The present findings call for the further studies to isolate the hypoglycemic constituent (s) of the plant and elucidate exact mechanism of action.

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