Antidiabetic Activity of Methanol Extract and Fractions of *Thymus schimperi* Ronnier Leaves in Normal and Streptozotocin Induce Diabetic Mice

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ABSTRACT

To study the anti-diabetic effects of the crude leaves extract and fractions of *Thymus schimperi* Ronnier in normal and streptozotocin induced diabetic mice. The crude extract and the fractions were screened for antidiabetic activities in streptozotocin induced diabetic mice. A normoglycemic mice model and oral glucose tolerance test were also undertaken to assess the hypoglycemic and antihyperglycemic effect of the crude extract in normoglycemic and glucose loaded mice, respectively. The methanolic crude extract has significantly reduced blood glucose level in streptozotocin induced diabetic mice at all given doses compared to the negative control and the percentage reduction observed was in a dose dependent manner i.e. [250 mg/kg (14.76±6.1%), 500 mg/kg (25.12±11.5%) and 750 mg/kg (27.15±10.0%)]. The crude methanol extract was devoid of hypoglycemic effect in normoglycemic mice but significantly reduced post prandial hyperglycemia starting from 1 h post glucose administration. Among the fractions, higher percentage reduction was recorded in the n-butanol fraction at a dose level of 500 mg/kg (36±7.3%) compared to 250 mg/kg (22.2±4.3%). The aqueous fraction 250 mg/kg and 500 mg/kg also reduced the blood glucose level by 17.6±6.0% and 18.4±5.0%, respectively. This study revealed that methanol extract as well as butanol and aqueous fractions of *T schimperi* possess anti-diabetic activity.

Conflicts of Interest: Declared None

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INTRODUCTION

Diabetes Mellitus is manifested by persistent hyperglycemia and other complications including the development of retinopathy, nephropathy, heart attack, stroke and peripheral arterial disease [1]. The current approaches to the management of diabetes mellitus include administration of insulin and/or oral anti-diabetic drugs together with lifestyle modification and dietary therapy [2]. However, these drugs are associated with many adverse reactions including gastrointestinal disturbance, blood dyscrasia, hypersensitivity reactions, and lactic acidosis [3]. Besides, a recent study indicated that up to 2.5% and 17.5% of sulphonyl urea treated patients experience major and minor hypoglycemia, respectively [4]. Body weight increases of 1 to 5 kg are also common with both sulphonylurea and tiazolidindiones therapy. Hence, there exists a huge need, among the scientific community, to develop new anti-diabetic drugs. With this regard, it’s believed that medicinal plants could be potential sources of anti-diabetic drugs.

*Thymus schimperi* Ronnier (Fam. Lamiaceae) is a
perennial herb, locally known as Tosign in Amharic and is
demically found in Ethiopia. An ethnobotanical study
ported that T schimperi has been traditionally used for the
tement of diabetes in Ethiopia [5]. So far, the plant was
armacologically evaluated for its diuretic, anti-
 hypertensive effect [6] and antimicrobial effect [7]. Besides,
other study showed the blood glucose lowering effects of
the leaves in alloxan-induced diabetes mice model [8]. The
aim of the present study was to investigate the antidiabetic
effects of the methanol leaves extract and its fractions (ethyl
acetate, n-butanol and aqueous) in streptozotocin induced
diabetic mice model. The herbal extract was also evaluated
for the hypoglycemic nature and post prandial glucose
lowering potential in normal and glucose loaded mice,
respectively.

MATERIALS AND METHODS

Animals

Swiss albino mice of either sex weighing 20-30g were
used with the approval of Research Ethics Committee of the
College of Health Sciences, Mekelle University (ERC
1028/2017). Animals were kept in plastic cages,
acclimatized to the laboratory condition at room temperature
under naturally illuminated environment of a 12 h light: 12 h
dark cycle. Animals were fed a standard pellet and water was
given ad libitum.

Plant materials and preparation of extracts and solvent
fractions

Thymus schimperi Ronniger (Lamiaceae) were collected
from Gudoberet, North Shoa Zone, Amhara region, Ethiopia.
The plant was authenticated and the voucher specimen was
deposited at the National Herbarium, Department of
Biology, College of Natural Sciences, Addis Ababa
University, Ethiopia (AS001/2016).

The plant leaves were dried under shade and crushed to
course powder using electrical grinder. The powdered leaves
(800g) were extracted with 80% methanol by maceration
method with a continuous shaking in orbital shaker for 6 h at
room temperature. The residue was removed by filtration
and the process was continued for three times till the marc
changes to colorless. A rotary evaporator was used to dry the
combined filtrate at 40ºC under reduced pressure. The
approximate yield of methanol extract was 14.63%. The
extract was administered orally after dissolving in distilled
water. The leaves extract of T schimperi was screened for the
presence of phytochemicals and showed positive result for the
alkaloids, phenolic compounds, flavonoids, saponins,
terpinoid and tannins. 30g of methanol leaves extract was
further fractionated using solvents in increasing polarity: n-
heptane, ethyl acetate and n-butanol. Thus the obtained
fractions were kept in glass bottles and stored in a
refrigerator for future investigation.

Acute oral toxicity test

Five female albino mice weighing 20-25 g were fasted
for 4 h and orally administered 2000 mg/kg b.w. of the
extract. Mice were observed continuously for the first 4 h
and then periodically up to 24 h for toxic manifestations like:
drowsiness, restlessness, writhing, convulsion, piloerection
and mortality if any and the observation was continued for
two weeks [9].

Induction of diabetes in mice

Diabetes was induced in mice as per the methods
previously described [10, 11]. Swiss albino mice were
allowed to fast for 8 hours and a single dose (150 mg/kg i.p.)
of streptozotocin dissolved in 0.1 M sodium citrate buffer
was injected to them. Blood glucose level of each animal
was determined after 72 hours and animals with a fasting
blood glucose range above 200 mg/dl were selected for the
study. These diabetic mice were divided into five groups,
each containing six animals (n=6): Group II, (untreated
mice) given 0.1 ml distilled water, Group III, diabetic mice
given 0.66 mg/kg glibenclamide, Groups IV, V and VI were
treated with 250, 500 and 750 mg/kg of methanol extract of
Thymus schimperi leaves respectively. Group I (normal
control) received 0.1 ml distilled water. Treatments were
given orally daily for fifteen days and their body weight and
blood glucose levels were measured at day 0, 5, 10 and 15.

Evaluation of hypoglycemic activity in normoglycemic
mice

Hypoglycemic effect of methanol extract of Thymus
schimperi leaves was evaluated on five groups of normal
mice (n=6) fasted for 8 hours but allowed free access to
water [11]. Group I (normal control) received (0.1 ml p.o.)
distilled water, Group II, received glibenclamide (0.66
mg/kg p.o.), Groups III, IV and V were treated with 250, 500
and 750 mg/kg of methanol extract of Thymus schimperi
leaves respectively. Blood glucose levels were assessed at 0,
½ h, 1 h, 2 h, and 3h intervals by using a One Touch
Glucometer (Prodigy Autocode, USA).

Oral glucose tolerance test

This test was undertaken based on the previously
described method with little modification [12]. Mice were
fasted for 8 hours and divided into six groups (n=6). First
two groups were received 0.1 ml distilled water orally.
Group III, treated with standard drug glibenclamide (0.66
mg/kg, p.o.), Groups IV, V and VI were treated with 250, 500
and 750 mg/kg of methanol extract of Thymus schimperi
leaves respectively. Glucose solution (2.5 g/kg, p.o.) was
administered orally to each group 30 minutes prior to the
administration of plant extract. Blood glucose levels were
estimated at 0, ½ h, 1 h, 2 h, and 3h of post glucose
administration using One Touch Glucometer.

Evaluation of antihyperglycemic effect of fractions in
streptozotocin induced diabetic mice

Animals were divided into nine groups (n=6) and the
fractions of Thymus schimperi leaves extract were given
orally. Group I, normal control received 0.1 ml distilled
water, Group II served as diabetic control and took 0.1 ml

Antidiabetic Activity of *Thymus schimperi* Ronniger Leaves

distilled water. Group III, positive control treated with glibenclamide (0.66 mg/kg) daily and each group of IV to IX were treated with two doses of each fraction (250 and 500 mg/kg) of ethyl acetate, n-butanol and aqueous, respectively. The animals were then observed for 15 days by measuring their body weight and blood glucose levels at day 0, 5, 10 and 15 [13].

**Statistical analysis**

Statistical analysis was done using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test. P value <0.05 was considered statistically significant. Data analysis was performed using SPSS software package Version 20.0.

**RESULTS**

**Acute oral toxicity study**

In the acute toxicity study, the methanol extract of *T. schimperi* leaves did not produce significant changes in behavior nor caused mortality up to a dose of 2000 mg/kg b.w. in female mice during the first 24 h as well as in the following 14 days of observation.

**Hypoglycemic effect in normal mice**

The blood glucose levels of the glibenclamide treated group showed statistically significant difference compared to the normal control group and the extract treated groups (p<0.05) (Table 1). The extract treated groups did not show any significant differences within 3 hours of treatment (p>0.05), whereas the positive control group showed statistically significant variation after 3 hour of administration (p<0.05).

**Oral glucose tolerance test in mice**

All extract treated groups significantly reduced the raised blood glucose staring 1 h post administration till 3 h, compared to the diabetic control group. Glibenclamide treated group showed the highest significant (p<0.05) reduction during this period (Table 2). The percentage reduction of blood glucose levels for 250, 500 and 750 mg/kg doses of extract at post 3 h administration of the extract compared with the blood glucose levels obtained at 30 minute post glucose administration of each group were 56.4, 49.16 and 46.98 %, respectively (Table 2).

**Effects on fasting blood glucose level of streptozotocin induced diabetic mice**

The fasting blood glucose levels of streptozotocin induced diabetic mice were increased (355.33±32.55) when compared to the normal control (160.00±14.40) (Table 3; Fig. 1). Besides, the fasting blood glucose levels were significantly reduced (p<0.05) at day 10 and 15 in diabetic mice treated with 500 mg/kg and 750 mg/kg methanol extract of *T. schimperi* where as those treated with 250 mg/kg showed a significant reduction only at day 15 (Table 3). The finding also revealed that the extracts showed a dose dependent percent reduction in fasting blood glucose levels: 14.76% (250 mg/kg), 25.12% (500 mg/kg) and 27.15% (750 mg/kg) on day 15 (Fig. 1).

The result of this study also showed that the aqueous fraction produced a significant reduction of fasting blood glucose levels at doses of 250 mg/kg and 500 mg/kg at day 15 and the butanol fraction showed a significant reduction in the fasting blood glucose levels at days 10 and 15 (Table 4). The peak percentage reduction (33.85%) was observed in the group treated with 500 mg/kg butanol fraction followed by the aqueous fraction at a dose of 500 mg/kg (19.04%). Aqueous fraction at dose 250 mg/kg reduced blood glucose by 17.49 % as well (Fig. 2).

**Effects on body weight of streptozotocin induced diabetic mice**

Table 1. Hypoglycemic effect of the crude leaf extract of *Thymus schimperi* in normoglycemic mice

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>BGL (mg/dl) during the treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Normal control</td>
<td>145.40±8.57</td>
</tr>
<tr>
<td>GB 0.66 mg/kg</td>
<td>163.80±5.98</td>
</tr>
<tr>
<td>TS250 mg/kg</td>
<td>172.20±3.37</td>
</tr>
<tr>
<td>TS500 mg/kg</td>
<td>175.00±14.70</td>
</tr>
<tr>
<td>TS750 mg/kg</td>
<td>166.00±4.34</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± S.E.M and all data were analyzed using ANOVA followed by Turkey’s test for multiple comparisons for means. n = 6, * when p<0.05 versus normal control (TS= *Thymus schimperi*, GB= Glibenclamide, BGL = Blood glucose level)

Table 2. The effect of the crude leaves extract of *Thymus schimperi* in glucose loaded mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>BGL (mg/dl) during the treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
</tr>
<tr>
<td>Normal control</td>
<td>149.20±7.10</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>161.20±8.05</td>
</tr>
<tr>
<td>GB 0.66 mg/kg</td>
<td>161.80±8.11</td>
</tr>
<tr>
<td>TS250 mg/kg</td>
<td>176.00±6.40</td>
</tr>
<tr>
<td>TS500 mg/kg</td>
<td>159.80±10.76</td>
</tr>
<tr>
<td>TS750 mg/kg</td>
<td>151.20±10.90</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± S.E.M and all data were analyzed using ANOVA followed by Turkey’s test for multiple comparisons for means. n = 6, * when p<0.05 versus negative control (TS= *Thymus schimperi*, GB= Glibenclamide, BGL = Blood glucose level)
In this study, the negative control showed 13% loss in body weight and the normal control group showed a 24.67% increase during the treatment period (Fig. 3). The body weight of diabetic mice receiving glibenclamide showed a 15.21% improvement in their body weight. A dose dependent body weight increment was observed in diabetic mice treated with the different doses of the extract (250, 500, & 750 mg/kg): 9.79%, 9.99% and 11.67%, respectively (Figure 3). Similarly, a dose dependent body weight increment was also observed in diabetic mice treated with n-butanol fraction i.e. 17.24% (250 mg/kg) and 18.31% (500 mg/kg) (Fig. 4).

**DISCUSSION**

Diabetes mellitus is one of the rapidly growing endocrine disorders with major complications affecting populations living throughout the world [14]. The pathophysiological mechanisms are being scrutinized and the knowledge on heterogeneity and complexity of this disease is being advanced. Accordingly, the search for more appropriate therapy is also being under way. In line with that, traditional medicines are used substantially by diabetic patients across the globe [15] and medicinal plants have been identified to be a target for scientists to come up with newer and better therapeutic options in the future. Streptozotocin (STZ) was used to induce diabetes in the current study. The toxic effect of the chemical is with its metabolic products and the free radicals generated, which finally destroy the pancreatic β-cells by alkylating DNA, impairing mitochondrial system and inhibiting O-GlcNAcase. It is a nitric oxide (NO) donor

**Table 3. Effects of the crude extract of leaves of Thymus schimperi on BGL (mg/dl) in STZ induced diabetic mice**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>BGL (mg/dl) during the treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal Control</td>
<td>160.00±14.40</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>355.33±32.55</td>
</tr>
<tr>
<td>GB 0.66 mg/kg</td>
<td>326.00±45.01</td>
</tr>
<tr>
<td>TS 250mg/kg</td>
<td>337.50±32.61</td>
</tr>
<tr>
<td>TS 500mg/kg</td>
<td>363.67±41.73</td>
</tr>
<tr>
<td>TS 750mg/kg</td>
<td>345.00±39.58</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± S.E.M and all data were analyzed using ANOVA followed by Turkey’s test for multiple comparisons for means. n = 6, * when p<0.05 versus Diabetic control and **p<0.01 (TS= Thymus schimperi, GB= Glibenclamide, BGL = Blood glucose level)

**Table 4. Effects of fractions of leaves of Thymus schimperi in fasting BGL (mg/dl) of STZ induced diabetic mice**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>BGL (mg/dl) during the treatment period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Normal control</td>
<td>160.60±17.62</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>393.20±32.59</td>
</tr>
<tr>
<td>Positive control GB0.66 mg/kg</td>
<td>333.00±18.31</td>
</tr>
<tr>
<td>Ethyl acetate 250mg/kg</td>
<td>347.00±30.51</td>
</tr>
<tr>
<td>n-butanol fraction 250mg/kg</td>
<td>354.20±18.10</td>
</tr>
<tr>
<td>Aqueous fraction 250mg/kg</td>
<td>384.60±38.47</td>
</tr>
</tbody>
</table>

Results are expressed in Mean ± S.E.M and all data were analyzed using ANOVA followed by Turkey’s test for multiple comparisons for means. n = 6, * when p<0.05 versus Diabetic control and **p<0.01 (GB= Glibenclamide, BGL = Blood glucose level)
and it’s indicated that NO contributes for the degeneration of the Langerhans islets beta cells [16]. In the present study, STZ induced persistent hyperglycemia and brings about a significant weight loss in experimental mice and it was consistent with many other studies. The loss in body weight is due to the fact that polyphagic condition and loss of weight tends to promote excessive catabolism of fats and tissue proteins as an alternative energy source because of unavailability of carbohydrates [17]. In this study, oral administration of *T. schimperi* leaves crude extract and fractions consecutively for two weeks to diabetic mice improved body weight of mice, which is an implication of a relatively good control of the diabetic condition. The effect of the extract on fasting blood glucose levels (FBGL) revealed that the extract showed a time dependent decrease in blood glucose levels: 14.76% (250 mg/kg), 25.12% (500 mg/kg) and 27.15% (750 mg/kg). With regard to the fractions, a significant blood glucose reduction in both dose levels was observed with the n-butanol and aqueous fraction. This might be due to the ability of the two solvents to leach out the required antidiabetic phytoconstituents from the extract. Peak value observed by n-butanol fraction as compared to the aqueous fraction can be explained by its more content of the antidiabetic phytochemicals.

The reduction in blood glucose levels of ethyl acetate fraction treated mice was not significant (*p* > 0.05) at a dose of 250 mg/kg and 500 mg/kg and this might be the absence or insignificant content of the active phytochemicals responsible for antidiabetic effect in the ethyl acetate fraction. Therefore, it is plausible to state that the polar phytochemicals in the extracts of *T. schimperi* are responsible for its antihyperglycemic effect.
The current finding indicated that, the leaves extract of *T. schimperi* showed antihyperglycemic effect on STZ induced diabetic mice without causing hypoglycemia on normoglycemic mice unlike that of the standard drug glibenclamide. Hence, it is reasonably assumed that the mechanism of action of the extracts is different from the standard drug. The mechanism probably resembles those of insulin sensitizers that include promoting tissue glucose uptake, reducing hepatic glucose output, interfering with glucose metabolism and there by producing an antihyperglycemic effect without hypoglycemia.[18]

The phytochemical analysis of this plant indicated the presence of alkaloids, polyphenols, flavonoids, tannins, saponins and terpenoids. Formerly, it was reported that polyphenols, flavonoids, alkaloids, terpenoids from different plant origin demonstrated anti-diabetic effects in animal model of diabetes.[19] Flavonoids are implicated in diabetes treatment by reducing the aldose reductase, regenerating the pancreatic cells, enhancing insulin release, increasing calcium ion uptake,[20] delaying the gastric emptying rate and reducing active transport of glucose across intestinal brush border membrane.[21] Various studies also reported about their potential powerful antioxidant properties.[22, 23, 24]. On the other side, previous studies done on saponins have demonstrated a protective effect on pancreatic islet cells and increase insulin secretion.[24] Finally, alkaloids had also been found to have anti-diabetic effect.[20] These findings of the secondary metabolites might be implicated for the antidiabetic activity of *T. schimperi*.

Oral glucose tolerance test (OGTT) measures the body’s ability to use glucose, the body’s main source of energy and is used to diagnose prediabetes and diabetes.[25] In the current study, glucose loaded mice showed a significant increase in the blood glucose levels as compared to the normal mice since it is attributed to increased hepatic gluconeogenesis and abnormal utilization of glucose by tissues. Mice treated with glibenclamide and all dose levels of the extract had shown a significant reduction in blood glucose levels starting from 1 h post glucose administration. This result is consistent with other findings in that some plants have the ability to improve oral glucose intolerance. For example, aqueous extract of *Justicia Schimperiana* leaves[26], *Moringa oleifera* Lam[27], and *Caylusea abyssinica* Fresen[28] were found to have an ameliorating effect for glucose intolerance. The significant reduction of blood glucose levels in hyperglycemic mice treated with the three doses of the extract could suggest that there exists an improved glucose homeostasis and the normal homeostasis mechanism might be augmented by the phytochemicals present in the extract.

One of the effective strategies to control diabetes is to inhibit the activity of enzymes like α amylase enzyme and α-glucosidase, which are involved in breaking down of starch molecule in to glucose and maltose.[29] Thus, inhibitors of α-amylase and α-glucosidase delay the metabolism of carbohydrate in the small intestine and decrease the postprandial blood glucose levels in diabetic patients.[30] According to a recent study, polar extracts of *T schimperi* exhibited highest percentage of α-amylase inhibition activity in *in vitro* model.[31] This finding supports our current study and helps to claim that the antihyperglycemic effect of the plant extract could be due to an extra pancreatic mechanism though this requires further investigations. A similar species, *Thymus serpyllum* also demonstrated 50% inhibitory effect on α-glucosidase enzyme in an *in vitro* investigation which could also have a clue to our current investigation.[32]. Furthermore, it has been demonstrated that treatment with antioxidants improves impaired insulin-mediated glucose uptake in animal models.[13]. Also, a study showed that the major phenolic components in thyme extracts, particularly thymol and carvacrol, exerted higher antioxidant activity than the well-known butylated hydroxytoluene and α-tocopherol.[33]. With these prior studies, it is possible to suggest that the antihyperglycemic
activity of the extracts of *T. schimperi* in STZ-induced diabetes might be due to the improvement of insulin resistance against oxidative stress.

**CONCLUSION**

This study revealed that the methanol leaves extract, and n-butanol and aqueous fractions possess antihyperglycemic effect in diabetic mice model justifying the local use of *T. schimperi* leaves in the treatment of diabetes.

**Ethics approval and consent to participate**

All animal procedures and experimental protocols were approved by the Research Ethics Committee of the College of Health Sciences, Mekelle University (with protocol number ERC 1028/2017).

**Availability of data and materials**

Corresponding author can provide all the datasets used in this particular study upon request. The plant material is freely available at the National Herbarium, Department of Biology, College of Natural Sciences, Addis Ababa University, Ethiopia.

**Funding**

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**ACKNOWLEDGMENTS**

We would like to acknowledge Mekelle University and Amhara Regional Health Bureau for sponsoring this study. We are most grateful to Debre Birhan Health Sciences College for allowing us to perform part of the experiment in their laboratories as well as Addis Ababa University National Herbarium for authenticating the plant species.

**CONFLICTS OF INTEREST**

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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