

In vivo Antidiabetic Activity Evaluation of Aqueous and 80% Methanolic Extracts of Leaves of *Thymus schimperi* (Lamiaceae) in Alloxan-induced Diabetic Mice

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Introduction: Diabetes mellitus disorder characterized by increase in serum glucose level as a result of change in fat, protein metabolism, and carbohydrate. The aim of the present study was to investigate the effects of the aqueous and hydroalcoholic leaf extract of *Thymus schimperi* on blood glucose levels.

Methods: The aqueous and 80% methanol extracts of *T. schimperi* leaves were prepared. Swiss albino mice of either sex weighing 20–30 g were selected for the experiments. Mice that were made diabetic were divided into seven groups to study the antihyperglycemic effect of the extracts. Diabetes was induced by single intraperitoneal injection of alloxan monohydrate (180 mg/kg body weight).

Results: After diabetic mice were treated with an extract of solvent at doses of 250 and 500 mg/kg for 21 days, there were significant decreases in fasting blood glucose when compared to diabetic controls. The observed antidiabetic activity could be associated with the phytochemicals present in this plant extract. The extract of solvent also prevented body weight loss of diabetic when compared to diabetic mice group. It was also observed that the extracts have shown no acute toxicity at a dose of 2 g/kg.

Conclusion: The aqueous and 80% methanol extracts of *T. schimperi* leaves have shown blood glucose level lowering effects in diabetic mice. Hence, the present study might support the traditional use of *T. schimperi* for diabetes mellitus treatment.

Keywords: diabetic, antidiabetic, alloxan, in vivo, antihyperglycemic, *Thymus schimperi*

Introduction

Diabetes mellitus (DM) is a metabolic disorder which is characterized by a persistent rise in blood glucose level (BGL) as a result of change in macromolecules such as fat, carbohydrate, and protein metabolism.¹ The two major pathophysiologies of DM are impairment of insulin action and secretion.² Type 1 DM and type 2 DM are the two most common forms of DM.³ Currently DM is becoming a leading public health problem globally. The World Health Organization (WHO) report nine percent (9%) of adults living in both developing and developed countries suffer from diabetes.⁴

Early diagnosis is the best approach to improve the overall health outcome of diabetic patients. Anyone who is diagnosed with diabetes, can utilize many lifestyle interventions including dietary management and regular exercise to manage their

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health status.⁵ In addition, the use of alternative and complementary medicine to the current therapeutic options of DM management have been widely promoted in order to manage this worldwide health problem.⁶

Even though varieties of pharmacological agents are proposed to control the elevation of BGL, the prevalence of DM is still rising and the health of societies is being endangered.⁷ Plants may act on BGL through various mechanisms such as blocking potassium channel of beta cell of pancreatic, adrenomimeticism, stimulation of secondary messenger; providing essential heavy metals for the pancreatic beta cells, inhibition of α -glucosidase and β -galactosidase, inhibiting free radical generation which may have a role in insulin secreting cell dysfunction found in DM,^{8,9} activation of glycolysis, citric acid cycle and glycogenesis and block of gluconeogenesis and glycogenolysis;¹⁰ enhancing efficacy of insulin, blocking insulinase enzymes activity, acting as insulin-like substances and increasing the number and/or efficacy of the beta cells of the pancreas through improving regeneration of beta cells to improve insulin activities. Secondary metabolites of herbs may also delay carbohydrate absorption; thus reducing glucose in the blood.¹¹ However, traditional medicine (TM) in Ethiopia has gained very little consideration in current medical research. Moreover, fewer struggles have been made to advance the contributions of TM practice in management of diseases and identification of side effects that may be associated with the use of plant medicines.

Thyme leaves are used to sweeten and flavor variety of foods in Ethiopia.^{12,13} A phytochemical analysis of the essential oil from *Thymus Schimperii* has demonstrated that terpinoids such as β -myrcene, α -terpinene, γ -terpinene, α -terpineol, limonene, carvacrol, α -humulene, p-cymene are the major constituents.^{14,15} In addition, the Ethiopian *T. Schimperii* has many traditional medicinal applications.¹³ *T. schimperii* is suggested for many uses based upon its antimicrobial (against bacteria, virus, fungus and others), spasmolytic and anti-tussive and scavenging free radical activity.¹⁶ The dried leaves of *T. schimperii* are also used in TM for management of gonorrhoea, relief of pain due to headache, toothache, stomachache, and earache, reduce inflammation, as antispasmodic, blood clotting, overcome urinary retention problems, mental illness like psychosis, eye disease, liver disease, respiratory disorder (leprosy and lung tuberculosis), skin disorder (acne

and ascaris).^{17,18} Some of pharmacological research on crude and fractional extracts of different parts of *T. schimperii* includes, the antioxidant activity and preservative effect,¹⁹ antimicrobial,²⁰ diuretic and antihypertensive activity of leaf extracts²¹ and antioxidant and α -amylase inhibition activities.¹² In particular in the Ethiopian Oromia region, North Shoa Zone, *T. schimperii* leaves have been used for the management of DM.²² Therefore, based on this TD value claim the current work is initiated to assess the antidiabetic activity of crude leaf extract of *T. schimperii* in chemically induced diabetic mice (acute study and subacute).

Materials and Methods

The instruments, reagents and drugs used in this study are Rotary evaporator (Buchi model R-200), Lyophilizer (Operan, Korea Vacuum Limited), digital weighing balance (Mettler Toledo model), deep freezer, one touch basic glucometer (Prodigy Autocode blood glucose monitoring system, Taiwan), strip (Prodigy blood glucose test strip, Taiwan) and mice cages. Methanol absolute (Blulux, India, Purchased from ZAF pharmaceuticals Pvt., Ltd, Co.), 530% of glucose solution, Tween 80% (BDH Laboratory Supplies Poole, BH151TD lot ZA2088516, England), sulfuric acid (Park Scientific Ltd, Lot 8114/10, UK), acetic anhydride (Techno Pharmchem, India), ferric chloride (Hopkin and Williams Ltd, England), potassium iodide BP (Evans Medical Ltd, England), Wagner's reagent, lead acetate, acetic anhydride, chloroform, normal saline (IV infusion BP Medsol pharmaceuticals) glibenclamide, and alloxan (Sigma Chemical Company).

Plant Materials Collections and Preparation

Fresh *T. schimperii* leaves were collected from Suluta, Oromia Region of Ethiopia located at 910' 59.988" N and 3845' 0.000" E and about 18 km north of Addis Ababa (capital city), Ethiopia. Then plant materials were cleaned and validated by a taxonomist at Ethiopian Public Health Institute (EPHI) and kept with voucher specimen GM-001 in the herbarium for future reference. Fresh leaves were washed with water from unnecessary materials, dried at room temperature, and size reduced by manual crusher to obtain the appropriate particle size that facilitate the extraction process.

Animals for Experiment and Study Protocol

Swiss albino mice weighing 20–30 g, six to nine weeks old, of both sexes were used with the approval of Research Ethics Committee of the College of Health Sciences, Addis Ababa University. The animals were obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa. All experimental animal protocols were in agreement with the standards set for the care and use of experimental animals by Committee for Purpose and Control of Supervision of Experiments on Animals, and approved by the Department of Pharmacology Research and Ethics Review Committee.²³ After randomization in to various groups and before initiation of experiment, the mice were acclimatized to animal house conditions for a period of seven days before experimentation to minimize effects of environment-induced physiological, cardiovascular, immune, central nervous and endocrine system changes due to stress associated with transportation.²⁴ Mice were kept in cages of suitable size, lined with easy wood serving as bedding (changed every 24 h), with natural night-daytime exposure and at room temperature. Before and during the experiment, the mice were allowed free access to standard mice pellets, made from ground animal food and tap water regularly. All the animal experiments were conducted at EHRI. Dose choice was dependent on single dose oral acute toxicity test, previous study of the crude aqueous extract,²¹ as well as pilot experiments.

Preparation of Extracts of *T. Schimperii* with Solvents

The aqueous extract was prepared by macerating crushed leaves of *T. schimperii* (400 g) by using distilled water for 72 h in an Erlenmeyer conical flask with frequent agitation using a mini orbital shaker adjusting at 170 revolutions per minute for 90 min at room temperature. The first extract was filtered using folded gauze and a nylon cloth. Then, the extract was filtered by Watman filter paper No.1 under pressurized suction filtration system and the marc was again macerated twice with the help of the same volume of water to deeply extract the leaves of plant material. Then filtrates from each extraction were combined. Then the filtrates were frozen overnight using a deep freezer and set water free by using a lyophilizer and the freeze-dried product was kept in desiccators until utilized for the experiment purpose.

Six hundred gram powders of *T. schimperii* leaves were macerated with 1000 mL of 80% methanol for three days in an Erlenmeyer conical flask with frequent agitation using a mini orbital shaker adjusting at 170 revolutions per minute for 90 min at room temperature. First extract was filtered using folded gauze and a nylon cloth. Then, the extract was filtered by Watman filter paper No. 1 using pressurized suction filtration system and the residual was macerated twice using equal volume of methanol to thoroughly extract the *T. schimperii* leaves constituents. Then filtrates from each extraction were combined and methanol was removed by evaporation using rotary evaporator at 40°C and the filtrates were frozen overnight by the help of deep freezer and set water free by using a lyophilizer. The final yield found was 57 g (9.5% w/w) it was stored in refrigerator at 2–8°C and fresh stock solution was prepared and used for the experiment.

Induction of Experimental Diabetes

Swiss albino mice of both sexes were fasted overnight and their weight and fasting blood glucose level was recorded. Alloxan was first weighed individually for each animal according to their body weight and then dissolved with 0.9% (w/v) normal saline just prior to injection and injected to overnight fasted mice through IP administration at a dose of 180 mg/kg/body weight to induce experimental diabetic mice. Food and water were presented to the animals 30 min after drug administration.²⁵ In order to prevent hypoglycemic shock and mortalities during hypoglycemic phase, 10% glucose in tap water was given via water bottle for next 24 h. Seven days after alloxan injection, plasma glucose level of each animal was determined and animals with a fasting blood glucose range above 200 mg/dL²⁶ were included in the study. The blood samples were collected from the tail of the mice.

Extracts Administration to Induced Diabetic Mice

Orally 250 and 500 mg/kg of the hydroalcoholic and aqueous extracts of *T. schimperii* were administered to Alloxan-induced diabetic mice (test groups); 0.66 mg/kg glibenclamide to positive control group; and distilled water (10 mL/kg) to negative control group. Normal control group (none diabetic mice) mice were received distilled water (10 mL/kg) orally. Mice in all groups were administered once daily for 21 days. BGL was measured by draining blood from the tail of each mouse. In the acute

experiments samples were collected at 0, 2, and 4 h post administration. In the chronic experiments, BGL was measured at weekly for three weeks. The animal fasting glucose levels were estimated on days 1, 7, 14 and 21.

Body Weight Determination

All groups of mice's body weight were documented before treatment (day 0) and throughout the treatment period (on days 7, 14 and 21). An appropriately adjusted electronic balance was used for measuring body weight of the experimental mice.

Acute Toxicity Test Extract of *T. Schimper* Leaves

Acute oral toxicity from extract of *T. schimper* leaves was evaluated in female mice (25–30 g), as per the Organization for Economic Co-operation and Development, Guideline 425, adopted on 3 October, 2008 guideline (OECD).²⁷ The mice were fasted overnight and the weight of each mouse was recorded just before use. Animals were divided randomly into a control and two treatment groups, each group consisting of five female mice. Negative control group received the vehicle and the left treatment groups were orally administrated a single dose of 2000 mg/kg per body weight of *T. schimper* leaves of 80% methanol and aqueous extract of maceration method. Mice were strictly observed for the first four hours following the administrations, and then once daily during the following 14 days. The neurological, behavioral and autonomic changes, physical changes like motor activity alertness, convulsions, coma, restlessness, diarrhea, lacrimation and appearance of the animals, changes in respiratory circulation, eyes, sleep and the like^{28,29} were

critically observed every day for 14 days for any alteration shown by mice.

Statistical Analysis of the Results

SPSS Version 20 software was used for analysis of the results of the study. The results were then communicated as mean \pm standard error of the mean (\pm SEM). Differences between means of all parameters were done using ANOVA. Then, the Tukey's post-hoc tests with multiple comparisons were followed to determine the source of significant differences. Statistically A value of $P < 0.05$ was considered statistically significant. Extract treated groups were compared to positive control, negative control and standard control.

Results

The current study was done to evaluate the antihyperglycemic activity of methanol 80% and aqueous extract of *T. schimper* leaf in alloxan-induced diabetes mice. In the preparation of crude aqueous extract from the dried leaves of *T. schimper* a yield 10.7% was obtained. In the case of crude 80% methanol extract preparation, a yield of 13.2% of *T. schimper* was obtained.

Result of Crude Extract of *T. Schimper* on Diabetic Mice After Acute Treatment

The effect on BGL in alloxan-induced mice after oral administration of the methanol 80% and aqueous extract of *T. schimper* at different doses displayed a reduction in BGL in a time and dose dependent manner (Table 1). Treatments with *T. schimper* aqueous extracts of 250 and 500 mg/kg indicated reduction in 22.65 and 33.15% in plasma glucose levels, respectively after four hours of extract

Table 1 Effects of Aqueous and 80% Methanol Extract of *Thymus Schimper* on Diabetic Mice After Acute Treatment

Group	Treatment (mg/kg)	BGL (mg/dl)			
		0 h	2 h	4 h	% of Reduction from 0–4 h
I	NC	104.2 \pm 3.1	101.00 \pm 2.7	96.2 \pm 3.5	7.7
II	NS (10 mL/kg)	345.6 \pm 13.5	363.40 \pm 10.8	375.6 \pm 10.6	–8.7
III	GL (0.66)	329.8 \pm 36.7	197.00 \pm 26.3*	170.8 \pm 25.2**	48.2
IV	AE 250	319.6 \pm 59.6	278.00 \pm 60.6	247.2 \pm 60.4	22.7
V	AE 500	330.00 \pm 35.1	250.60 \pm 26.7	220.6 \pm 34.1	33.2
VI	MeOH 250	328.6 \pm 55.3	272.60 \pm 53.9	230.0 \pm 55.1	30.1
VII	MeOH 500	331.60 \pm 35.7	252.40 \pm 31.9	204.4 \pm 26.9*	38.4

Notes: NS: diabetic control (receiving distilled water 10 mL/kg), GL: diabetic control (receiving glibenclamide 10 mL/kg); AE250: aqueous extract 250 mg/kg; AE500: aqueous extract 500 mg/kg; ME250: methanol extracts 250 mg/kg; ME500: methanol extracts 500 mg/kg. *Significant values at $P < 0.05$ compared to group II. **Significant values at $P < 0.01$ compared to group II.

Abbreviation: NC, normal control.

administration. In case of 80% methanol extraction at 250 and 500 mg/kg indicated reduction of 30.06 and 38.35% in plasma glucose levels, respectively after four hours of extract administration. The 500 mg/kg of 80% methanol extract indicated a significant reduction in the BGL while both aqueous extract (250 and 500 mg/kg) and other dose of 80% methanol extract (250 mg/kg) showed no significant effect when compared to positive (diabetic) control group at four hours after extract administration. Glibenclamide (0.66 mg/kg) produced a significant reduction of 48.21% in the BGL after four hours of drug administration.

Result (Effect) of Extract of *T. Schimper* on BGL in Diabetic Mice After Prolonged Treatment

A noticeable increase in fasting BGL was obtained in chemically (alloxan)-induced diabetic mice compared with the normal control group. According to one-way ANOVA analysis there was significant difference among diabetic control and the group that received the standard drug (Table 2). Analysis of post hoc test showed that aqueous (500 mg/kg) and 80% methanol extract (500 mg/kg) showed significant drops in the BGL compared to diabetic control ($P<0.05$) at 14 days. In addition, a similar result was detected at 21 days to that at 14 days, but there was significant decrease in BGL in 80% of methanol extract (500 mg/kg) ($P<0.001$) and also significant decrease in BGL for aqueous 250 mg/kg and 80% methanol extract at dose 250 mg/kg ($P<0.05$) compared to diabetic control. However, glibenclamide and *T. Schimper* treatments failed to bring the blood glucose levels to normal values as in the nondiabetic control mice.

Effect of *T. Schimper* on Body Weight in Chemically Induced Diabetic Mice

At the end of experimental study, body weights of mice in normal control group (nondiabetic) were increased compared to their original body weights while in the diabetic control group (diabetic mice without any treatment intervention) a significant drop in the body weight was observed, when their final body weights were compared with their initial body weights. Alloxan-induced diabetic mice displayed significant decline in body weight compared to normal mice as shown below in Figure 1. Aqueous and methanol extract of two doses (250 and 500 mg/kg body weight) treated groups mice indicated enhancement in body weight when compared to diabetic mice in control group, however, it was still less than in the normal control group. Similarly the body weights of the alloxan-induced diabetes+glibenclamide group were also increased significantly.

Acute Toxicity Study

The present study conducted as per the OECD guideline 425 revealed that aqueous and methanol extracts of *T. schimper* did not yield any morbidity during the study period of 14 days at dose of 2 g/kg of body weight. No neurological, behavioral and autonomic changes, physical changes like motor activity alertness, convulsions, restlessness, diarrhea, coma, lacrimation and appearance of the animals and no changes in respiratory circulation, eyes, sleep and the like were observed throughout study period. Moreover, the *T. schimper* leaves extract did not lead to death in the mice at a dose of 2 g/kg during the study period time. The finding indicated that a single dose of *T. schimper* extracts of both solvents had no adverse

Table 2 Effects of Aqueous and 80% Methanol Crude Extracts of *Thymus Schimper* on the Blood Glucose Level in Alloxan-induced Diabetic Mice After Prolonged Treatment

Group	Treatment (mg/kg of Body Weight)	BGL (mg/dL) (Mean \pm SEM)			
		Day 0	Day 7	Day 14	Day 21
I	Normal control	97.5 \pm 3.57**	98.8 \pm 2.5**	102.0 \pm 2.9**	99.3 \pm 4.1**
II	NS (10 mL/kg)	324.8 \pm 19.6	340.3 \pm 20.9	339.5 \pm 21.7	328.8 \pm 23.1
III	GL (0.66)	270.5 \pm 32.4	218.0 \pm 36.7*	195.3 \pm 30.8**	145.8 \pm 7.7**
IV	AE 250	269.0 \pm 45.0	254.8 \pm 41.5	238.0 \pm 39.2	226.8 \pm 39.2*
V	AE 500	265.3 \pm 13.5	247.5 \pm 8.2	227.3 \pm 10.5*	209.5 \pm 8.3*
VI	MEOH 250	274.3 \pm 28.7	258.3 \pm 30.7	230.0 \pm 28.5	206.8 \pm 29.8*
VII	MEOH 500	270.3 \pm 13.2	239.0 \pm 12.9	215.8 \pm 13.1*	195.5 \pm 13.1**

Notes: Statistical significant test for comparison was done by ANOVA, followed by Tukey's test. Data are expressed as SEM; n=6. *Significant values at $P<0.05$ compared to group II. **Significant values at $P<0.01$ compared to group II.

Abbreviations: NS, normal saline; GL, glibenclamide; AE, aqueous extract; MEOH, methanol extract.

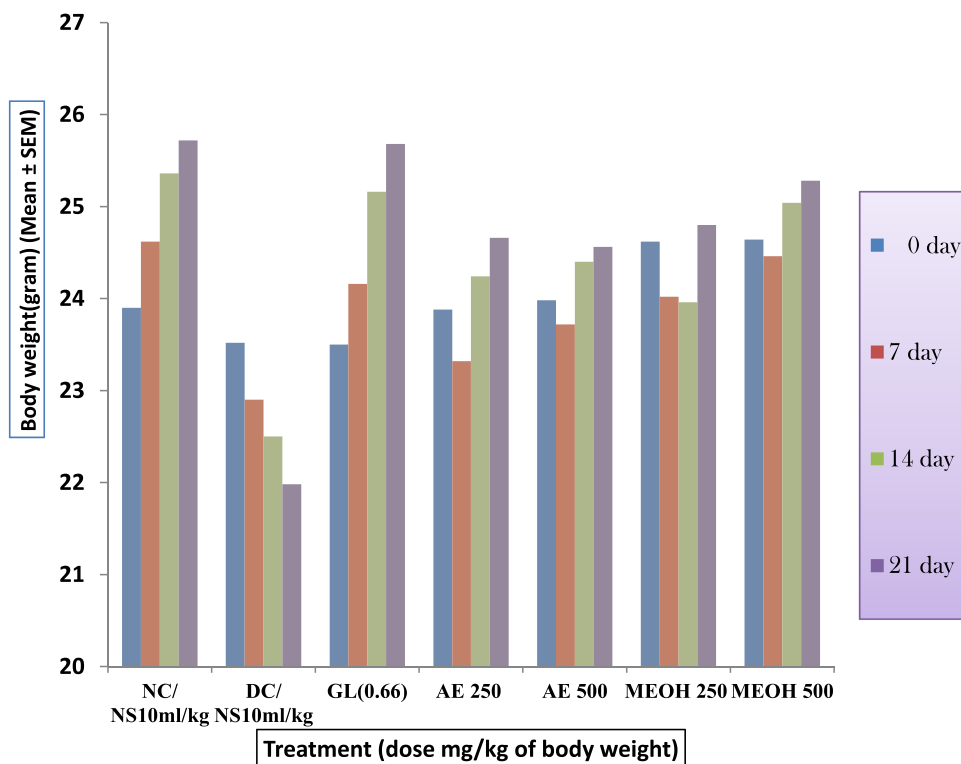


Figure 1 Effects of aqueous and 80% methanol *Thymus schimperi* leaves extract on body weight.

Notes: AE250: aqueous extract 250 mg/kg; AE500: aqueous extract 500 mg/kg; MEOH250: methanol extracts 250 mg/kg; MEOH500: methanol extracts 500 mg/kg; NS: 0.9% normal saline

Abbreviations: GL, glibenclamide; NC, normal control; DC, diabetic control.

effect, demonstrating that the LD₅₀ could be greater than 2 g/kg/body weight in mice.

Discussion

Use of *T. schimperi* in diabetes has been reported in the literature along with several other traditional claims. In order to establish the scientific evidence for the utility of *T. schimperi* in the management of DM, experimental study of the antihyperglycemic effects of the crude 80% methanol and aqueous leaves extracts were performed in alloxan-induced diabetic mice. The increase in fasting blood glucose (FBG) concentration is an important characteristic feature of DM.³⁰ In this work the extract of *T. schimperi* leaves lowered FBG level in diabetic mice. The aqueous and the 80% methanol extract of *T. schimperi* leaves (dose: 250 and 500 mg/kg body weight) were administered daily for three weeks to alloxan-induced diabetic mice. In this work, from around the end of the second week to the final week of treatment, significant fall of the BGL of the mice was observed gradually. Therefore, the current study showed that both the aqueous and the methanolic extracts of *T. schimperi* leaves have a

significant antidiabetic effect on alloxan-induced diabetic mice in a time and dose dependent manner.

The result of the study indicated the potential antihyperglycemic effect of the extract. There are many possible explanations related to this finding. The inhibition of human digestive tract pancreatic α -amylase activity describes one of the treatment methods commonly used for the prevention and control of hyperglycemia in type 2 DM patients after food intake by dropping the absorption of glucose released by this enzyme from starch.³⁰ Thus inhibition of this enzyme involved the delaying of postprandial hyperglycemia.³¹ In a study done by Dessalegn et al, *T. schimperi* confirmed the α -amylase inhibitory effect in vitro¹². Therefore, this property might be contributed to the antihyperglycemic activity of *T. schimperi* leaves extract, which supports our study.

In addition, it has been suggested that diabetic complications can be alleviated by the use of antioxidants through reduction of oxidative stress.³² Alloxan induce DM by destruction of pancreatic beta cells selectively via disruption of the cell membrane integrity. One of the mechanisms for its cell destruction is by intracellular

generation of free radicals and this supported the study on the antioxidant property of *T. schimperi* leaves in vitro and reported as the plant has the claimed activity.¹⁹ Therefore this property of the studied plant might have a role in controlling hyperglycemia of DM patients. Thus *T. schimperi* may have a role in control of DM.

Furthermore, *T. schimperi* may prevent the destruction or regenerated leftovers of the already alloxan-destroyed beta cells of pancreas³³ by one of its secondary constituents, polyphenolic, and contribute in significant BGL effect.³⁴ Thus, the antidiabetic result of the *T. schimperi* extract may be associated with the existence of different secondary metabolites in plants. One study described the antidiabetic result of thymus plants is due to be due α -glucosidase enzyme inhibitory effect and antioxidant (free radical scavenger) activity in vitro of the plant.³⁵ The result of our study agrees with this.

Weight loss in DM patients is due to deficiency of insulin in the DM patient which leads to reduction in the level of protein synthesis due to declined amino acid uptake by tissues and resulting lipolysis in adipose tissues and protein breakdown.^{36,37} In this study, there is the weight reduction in diabetic control mice. However, after treatments of diabetic mice were with *T. schimperi* extract, the weight loss was normalized. The capability of *T. schimperi* extracts to defend weight loss may be due to the bioactive compounds of *T. schimperi* leaves, which suppresses the free radicals generated via hyperglycemia. It also controls the muscle loss resulting from poor glycaemic control in diabetic mice¹² and lead to normalize the level of body weight.

In the current study acute oral toxicity was tested and found to be safe at a dose of 2000 mg/kg per body weight. In the acute toxicity test of aqueous extract of *T. schimperi*, there were no any physical changes and zero mortality. This finding indicated the strong data of the nontoxic effect of the aqueous extract of *T. schimperi*.³⁸

Conclusion

From this study it was shown that in *T. schimperi* leaves crude extracts of both solvents (80% methanol and aqueous) showed significant reduction of BGL on diabetic mice and prevented body weight loss of diabetic. Methanolic extract showed a noticeable drop of blood glucose level related to respective doses of aqueous extract. Therefore, based on the results obtained from the antihyperglycemic study it is possible to say that *T. schimperi* leaves can be used as a substitute supplement for the

management of DM. Moreover, additional investigation is needed for explanation of the appropriate mechanisms of action of the leaf extracts and its fractions.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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