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Comparative hypoglycemic activity of different extracts of *Teucrium stocksianum* in diabetic rabbits

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Abstract

In the present study crude powder and various extracts of *Teucrium stocksianum* were evaluated for anti-diabetic effect in alloxan-induced diabetic rabbits. Crude extract significantly reduced blood glucose level of normal and diabetic rabbits. The results were comparable with standard drug glibenclamide. Ethyl acetate extract (500 mg/kg) produced maximum decrease in blood glucose level among all the extracts and was selected for further study. Ethyl acetate extract significantly inhibited rise in glucose level in normal rabbits after an oral glucose load. The extract showed synergistic effect with different doses of insulin. Serum insulin level of diabetic rabbits was also significantly increased by the extract. HbA1c level was significantly ($p<0.05$) reduced whereas hemoglobin level was significantly increased by the extract. It is concluded that ethyl acetate extract may be a good remedy to manage diabetes and its complications.

Introduction

Diabetes mellitus is a complex metabolic disorder characterized by hyperglycemia with most common signs and symptoms of polyuria, polydipsia and polyphagia. The pathophysiology of diabetes mellitus involves defects in insulin secretion or insulin action, or both (Akhtar et al., 2002). Chronic hyperglycemia during diabetes causes gyration of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries (Rao et al., 1999). Chronic hyperglycemia itself can induce reactive oxygen and nitrogen species production. In addition, hyperglycemia can lead to the mitochondrial electron transport chain overloading which results in generation of more superoxide ions causing the long-term damage, dysfunction and eventually the failure of organs especially the eyes, kidneys, nerves, heart and blood vessels (Zimmet et al., 2001). Hyperglycemia-induced glucose oxidation initiates membrane lipid peroxidation and non-enzymatic glycation of proteins which in turn lead to enhanced production of reactive oxygen species (ROS) or result in decreased efficiency of inhibitory and scavenging system (Nishikawa et al., 2000).

The currently available synthetic drugs for the management of diabetes produce severe adverse effects. Moreover, there is no anti-diabetic agent for the complete cure and control of complications associated with diabetes (Cui et al., 2010). The search for compounds with novel properties to deal with the disease condition is still in progress. Medicinal plants have been used for the treatment of various diseases since time immemorial. Plant derived drugs are of low cost and considered free from harmful effects. Herbal drugs are used on folklore basis for the management of diabetes throughout the globe. These medicinal plants are required to be scientifically evaluated before their use in modern therapy. *Teucrium stocksianum* Boiss, belonging to family Labiatae, has been traditionally used in the

treatment of diabetes in Northern hilly areas of Pakistan basis (Ali and Shah, 2011). So, it was thought worthwhile to evaluate the anti-diabetic activity of *T. stocksianum* in normal and alloxan-induced diabetic rabbits.

Materials and Methods

Plant material used

Aerial parts of the plant were collected from the hills of Talash District Dir (lower) of Malakand Division Khyber Pakhtonkhva, Pakistan in the month of April (2010). The plant was identified and authenticated by renowned taxonomist Prof. Jehandar Shah, Vice-Chancellor Shaheed Benazeer Bhuto University Sheringle Dir (Upper). After collection the unwanted part and other adulterant were removed and the plant material was completely dried under the shade and powdered finely with the help of herbal grinder. The powdered material was stored in well closed cellophane bags at 4°C in the refrigerator.

Animals used

Adult healthy rabbits of either sex of local bread (*Oryctolagus cuniculus*), weighing about 1.2-1.5 kg were used in the study. Animals were housed at standard conditions of temperature (23 ± 12°C), humidity (55 ± 15%) and 12 hours light (7.0-19.0). Animals were provided with a free access to a balanced rabbit's diet consisting of green leaves, fodder, pulses (*Medicago sativa*) and water *ad libitum*. All the rabbits were kept randomly into different groups (6-8 animals per group) that were used in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals in this study (Zaman and Ahmad, 2004).

Chemicals and diagnostic kits used

Glucose oxidase kits (Optium Xceed, Abbot Laboratories USA), HbA1c (Merck Chemical Co., Germany), alloxan monohydrate (Research Organics, USA), glibenclamide (Valor Pharmaceuticals (Pvt) Ltd Islamabad), insulin (Regular Humulin-Lilly, USA), ether, chloroform, ethyl acetate and methanol. All chemicals used were of analytical grade.

Biochemical analysis

Blood glucose level of normal and diabetic rabbits was measured with the help of glucometer using glucose oxidized optimum kits. ELISA reader was used for the estimation of serum insulin level. HbA1c were measured by enzymatic test kits using Microlab 3000.

Preparation of various extracts of T. stocksianum

Successive extraction was carried out through cold maceration by using five different solvents viz. ether, chloroform, ethyl acetate, methanol and distilled water. Briefly; one kg of the dried powder was taken and extracted with the mentioned solvent one by one on the basis of increasing polarity. The powder was first soaked in ether for 24 hours with occasional shaking, then filtered with muslin cloth and finally through filter paper. The same procedure was adopted for remaining solvent. All the extracts except aqueous extract were concentrated with the help of rotary evaporator. The extracts were dried by using lyophilizer. Dried extracts were stored in the sealed container at 4°C in refrigerator

Induction of experimental diabetes

After an overnight fasting, rabbits were made diabetic by intravenous injection of fresh solution of 150 mg/kg body weight of alloxan monohydrate (Akhter et al., 2002). After three days the blood glucose level of rabbits was measured and rabbits with blood glucose level between 250-300 mg/dL were considered diabetic and were used for further study (Olajide et al., 1999).

Hypoglycemic activity of crude powder in normoglycemic rabbits

The aim of this experiment was to evaluate hypoglycemic activity in normal rabbits. Briefly; adult rabbits of either sex were divided into four groups of six rabbits each. Group I served as untreated normal control and received 20 mL of 2% gum tragacanth solution. Group II and III were given orally 250 and 500 mg/kg body weight of powdered *T. stocksianum* suspended in 2% gum tragacanth solution respectively. Group IV was treated orally with 3 mg/kg body weight of glibenclamide. Blood glucose levels were checked at 0, 2, 4 and 6 hours intervals after administration of the crude powder and glibenclamide (Andrade-Cetto et al., 2005).

Hypoglycemic activity of crude powder in alloxan-induced diabetic rabbits

After an overnight fasting, diabetic rabbits were divided into four groups of six animals each. Group I serve as diabetic control and received 20 mL of 2%gum tragacanth, Group II received 3 mg/kg body weight of glibenclamide while Group III and IV received 250 and 500 mg/kg body weight of the crud powder of *T. stocksianum* respectively. All the drugs were administered orally suspended in 2% gum tragacanth solution. Blood glucose levels were measured at 0, 2, 4 and 6 hours intervals after the administration of crude powder.

Screening of different extracts of T. stocksianum for hypoglycemic activity in diabetic rabbits

Different extracts of *T. stocksianum* were tested for their hypoglycemic activity in alloxan-induce diabetic rabbits in order to screen out the extract that having a potent hypoglycemic activity.

For this experiment the overnight fasted rabbits were divided into six groups of six animals each. Group I served as untreated diabetic control and was received

20 mL of 2% aqueous gum tragacanth solution orally. Group II, received 500 mg/kg body weight petroleum ether extract, Group III received 500 mg/kg body weight of chloroform extract, Group IV received 500 mg/kg of ethyl acetate extract, Group V was administered 500 mg/kg body weight of methanolic extract and Group VI was treated orally with same dose of aqueous extract of *T. stocksianum*. Blood glucose levels were estimated at 0, 2, 4, 6 and 8 hours intervals after the administration of extracts.

Oral glucose tolerance test (OGTT) of ethyl acetate extract of T. stocksianum in normal rabbits

After screening of different extracts of *T. stocksianum* in diabetic rabbits, ethyl acetate extract showed maximum hypoglycemic activity which was further evaluated for oral glucose tolerance in normal rabbits.

The aim of this study was to evaluate the hypoglycemic effect of ethyl acetate extract of *T. stocksianum* in normal rabbits after the oral glucose load.

Briefly, the overnight fasted rabbits were divided into three groups of six animals each. Group I served as normal control and received orally 20 mL of 2% aqueous gum tragacanth solution. Group II was treated with 500 mg/kg body weight of ethyl acetate extract of *T. stocksianum* while Group III received glibenclamide 0.01 mg/kg body weight respectively. After 30 min of treatment, a loading dose of glucose 1 g/kg of body weight was given to all the three groups. The blood glucose levels of all the three groups were monitored at 0, 1/2, 1, 2, 3, 4 and 6 hours interval after oral glucose load (Perfumi et al., 1991).

Hypoglycemic activity of ethyl acetate extract of T. stocksianum with and without different doses of insulin in alloxan-induced diabetic rabbits

The aim of present study was to determine the synergistic effect of ethyl acetate extract of *T. stocksianum* with insulin in alloxan-induced diabetic rabbits. Briefly; overnight fasted rabbits were divided into five groups of six rabbits each. Group I was administered 6 units/kg body weight of insulin only. Group II, III and IV were administered one, three, two, and one units of insulin along with 500 mg/kg of ethyl acetate extract while, group-5 was administered ethyl acetate extract 500 mg/kg only. Blood glucose levels were estimated at 0, 0.5, 1, 2, 3, 4 and 6 hours interval after the administration insulin and ethyl acetate extract of *T. stocksianum* (Maqsood et al., 2009).

To study the effect of ethyl acetate extract of T. stocksianum on serum insulin level of diabetic rabbits

The aim of this study was to find out the effect of ethyl acetate extract of *T. stocksianum* on the serum insulin level of diabetic rabbits. Briefly, the rabbits were divided into three groups of six animals each. Group I and II served as normal and diabetic control and received orally 20 mL of 2% aqueous gum tragacanth solution receptively for 30 days. Group III served as treated and were administered orally 500 mg/kg of ethyl acetate extract of *T. stocksianum* for 30 days. Blood samples were collected from each group after 30 days and estimated for serum insulin level (Babu et al., 2003).

Effect of ethyl acetate extract of T. stocksianum on hemoglobin and glycosylated hemoglobin (HbA1c) levels in diabetic rabbits

The aim of this study was to evaluate the effect of ethyl acetate extract of *T. stocksianum* on the hemoglobin and glycosylated hemoglobin (HbA1c) levels in alloxan-induce diabetic rabbits for 90 days. Briefly the rabbits were divided into three groups of six rabbits each. Group I and two served as normal and diabetic control and received 20 mL of 2% gum tragacanth solution. Group III served as treated group and received 500 mg/kg of ethyl acetate extract orally for 90 days. After 90 days blood samples were collected from the jugular vein and estimated for hemoglobin and glycosylated hemoglobin (HbA1c) levels (Home et al., 2008; Knowler et al., 2002).

Results

The crude powder of *T. stocksianum* in a dose of 250 and 500 mg/kg reduced the blood glucose level significantly ($p<0.001$). However, a maximum decrease in blood glucose level occurred at 500 mg/kg of crude powder. Glibenclamide also significantly ($p<0.001$) reduced the blood glucose level. There was no significant change in blood glucose levels of control group receiving 2% gum tragacanth solution (Figure 1).

The crude powder of *T. stocksianum* significantly ($p<0.001$) decreased the blood glucose level of diabetic rabbits treated with both 250 and 500 mg/kg however, at a dose of 500 mg/kg maximum decrease in the blood glucose level occurred. Glibenclamide also significantly reduced ($p<0.001$) blood glucose levels of diabetic rabbits. There was no significant change noted in the blood glucose level of normal and diabetic control (Figure 2).

Ethyl acetate, methanolic and aqueous extract of *T. stocksianum* significantly ($p<0.01$) decreased the blood glucose level of diabetic rabbits at dose of 500 mg/kg however, a maximum decrease was noted with ethyl acetate extract of *T. stocksianum* as compared to methanolic and aqueous extract. There was no significant decrease in the blood glucose levels of the rabbits receiving chloroform, ether extract and diabetic control group (Figure 3).

Ethyl acetate extract of *T. stocksianum* significantly ($p<0.001$) inhibited the increase in the blood glucose

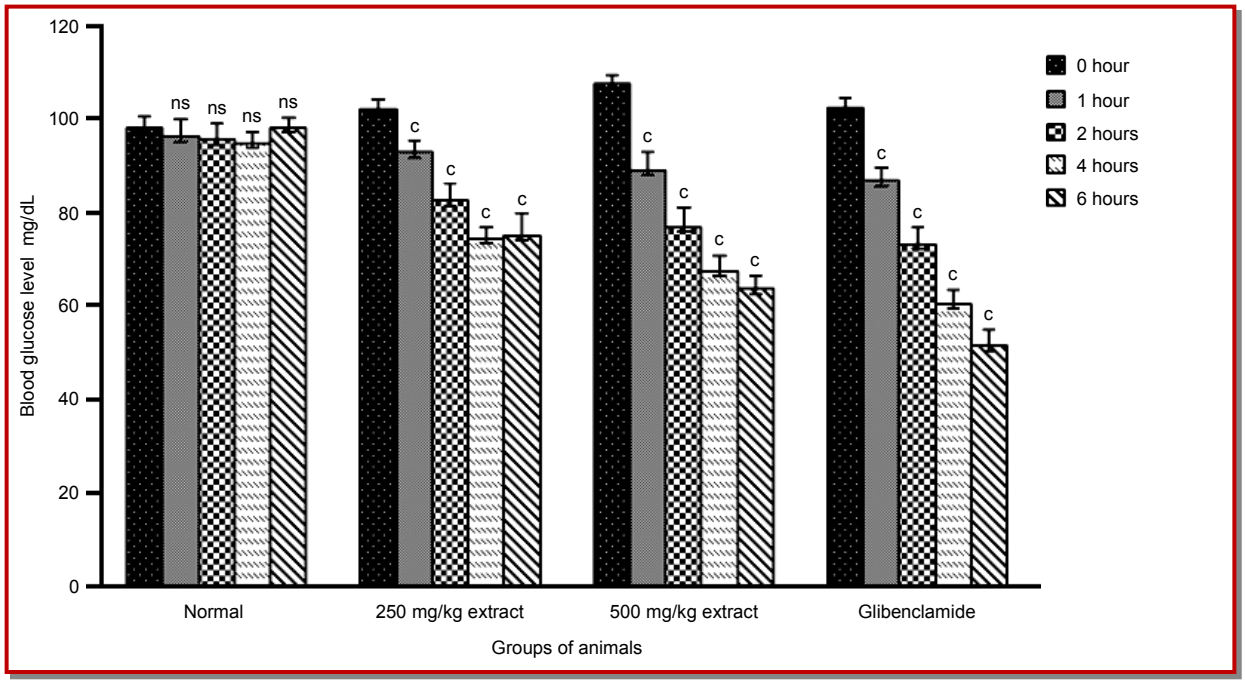

Figure 1: Evaluation of crude powder of *Teucrium stocksianum* for hypoglycemic activity in normoglycemic rabbits. (n = 6) where, ns = Non-significant change; cp<0.001

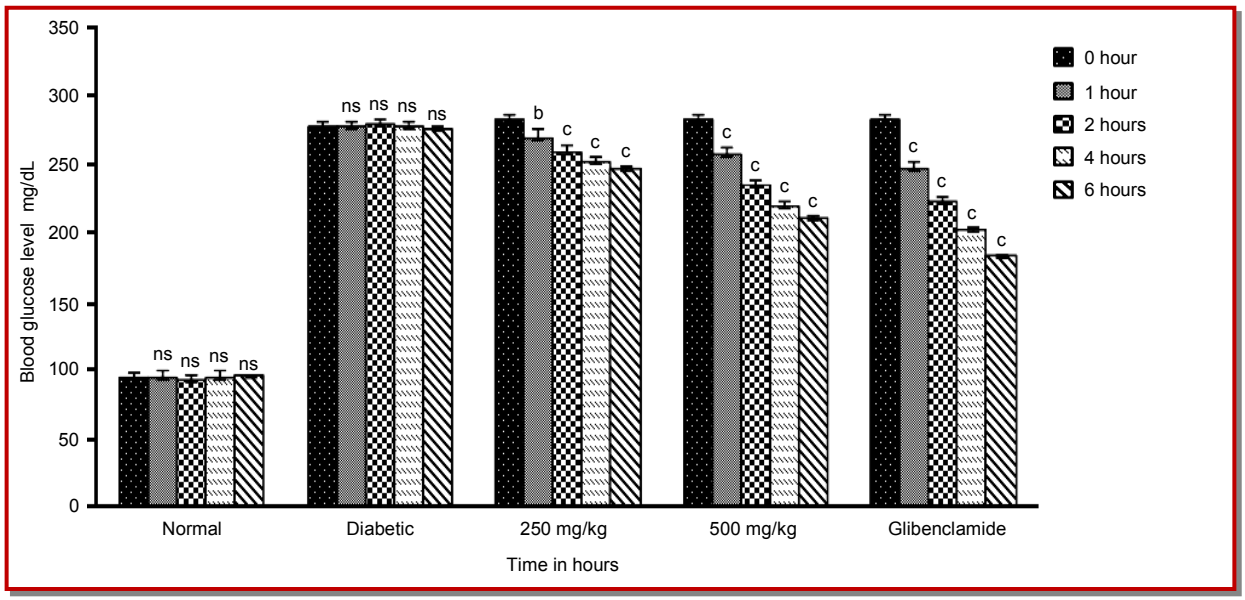

Figure 2: Hypoglycemic effect of crud powder of *Teucrium stocksianum* in diabetic rabbits. (n = 6) where, ns = Non-significant change; $^{b}p<0.01$; $^{c}p<0.001$ as compared to 0 hour

level after oral glucose load in normal rabbits. The glibenclamide also significantly (p<0.001) inhibited the increase in blood glucose level in normal rabbits. However, blood glucose level of normal control group of rabbits was significantly increased (Figure 4).

The ethyl acetate extract of *T. stocksianum* produced significant synergistic effect (p<0.001) with all doses of insulin used however, a maximum decrease in the blood glucose was observed when ethyl acetate extract of *T. stocksianum* was administered with 3 units/kg of insulin which was comparable to 6 units/kg of insulin alone (Figure 5). Ethyl acetate extract produced significant increase in serum insulin level of diabetic rabbits as compared to diabetic control group (Figure 6).

Ethyl acetate extract of *T. stocksianum* significantly (p<0.001) increased the hemoglobin level while significantly (p<.001) decreased the HbA1c level in diabetic treated rabbits during three month study as compared to diabetic control group (Figure 7).

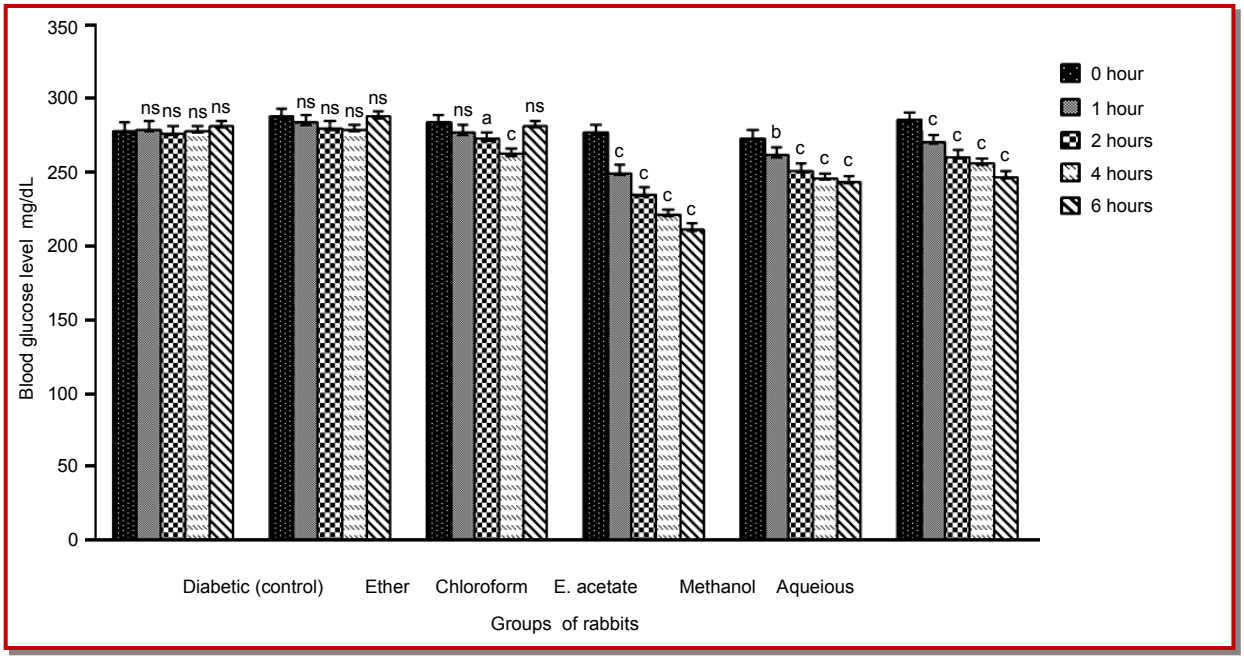

Figure 3: Hypoglycemic effect of different extracts of *Teucrium stocksianum* in diabetic rabbits. (n = 6) where, ns = Non significant change; [a]p<0.05; [b]p<0.01; [c]p<0.001 as compared to 0 hour

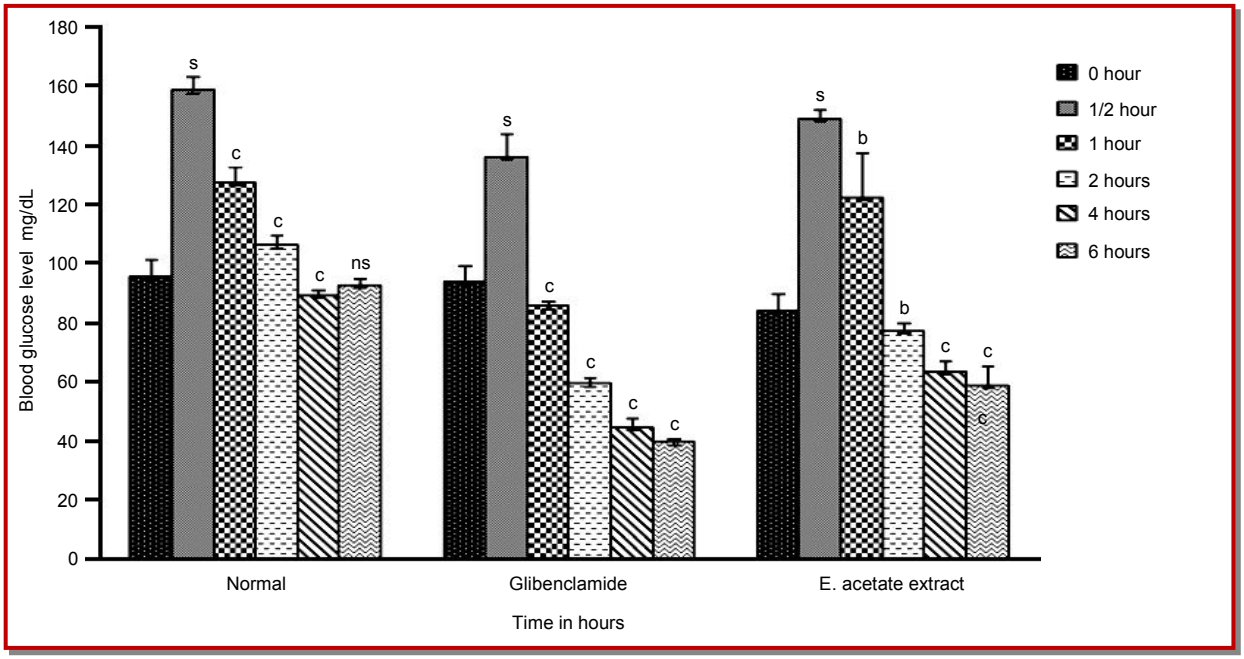

Figure 4: Oral glucose tolerance test (OGTT) of ethyl acetate extract of *Teucrium stocksianum* in normal rabbits. (n = 6) where, ns = Non significant change; [b]p<0.01; [c]p<0.001; s = significant increase (p<0.001) as compared to 0 hour

Discussion

Diabetes mellitus has been described as a metabolic disorder characterized by a high blood glucose level. In diabetes mellitus, β-cells of pancreas fail to produce sufficient insulin that leads to the increased blood glucose concentration (Goth, 1985). Currently diabetic management includes the use of insulin and various oral hypoglycemic agents such as sulphonylureas, biguanides, α-glucosidase inhibitors and glinides. The synthetic drugs are expensive and are reported to be associated with adverse effects. Search for more effective and safer antidiabetic agents is in progress and continued to be an important area of investigation. Traditionally considerable number of plants have been used as remedy for diabetes but their introduction into

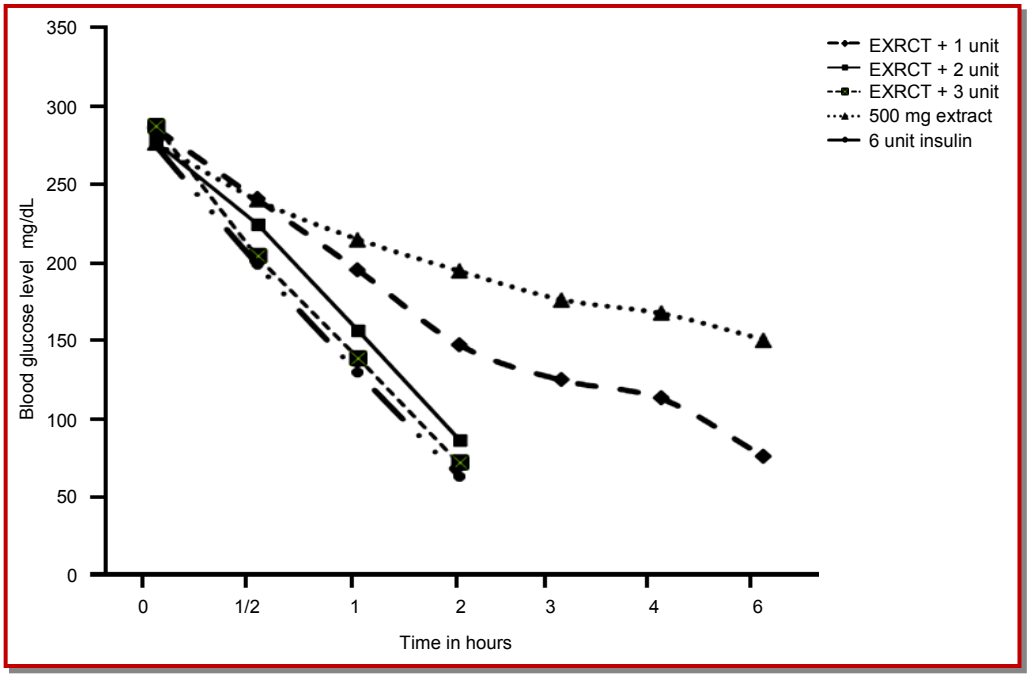

Figure 5: Hypoglycemic activity of ethyl acetate extract of *Teucrium stocksianum* with and without different units of insulin in diabetic rabbits

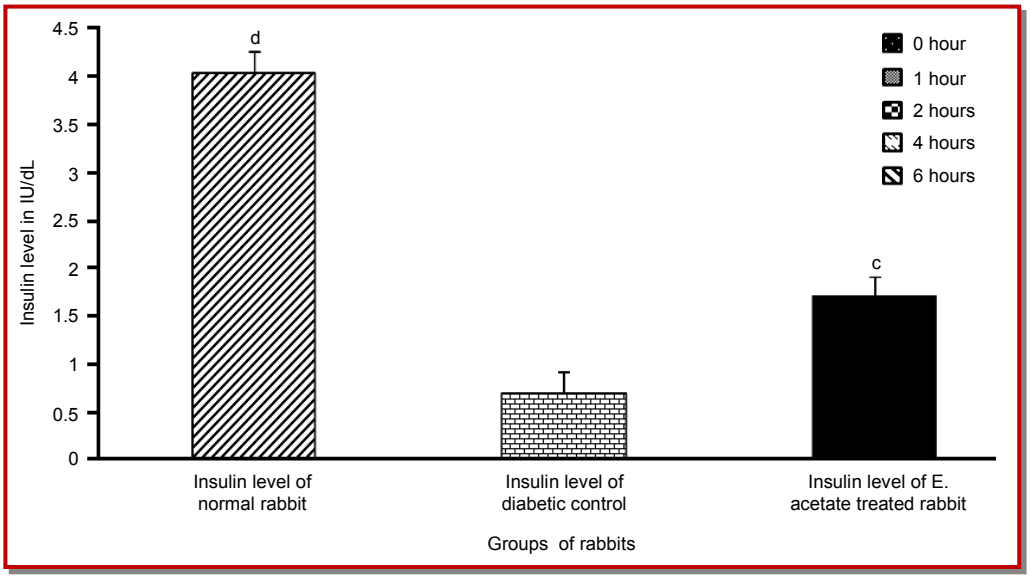

Figure 6: The effect of ethyl acetate extracts of on serum insulin level of diabetic rabbits 30 day, $^{c}p<0.001$; $^{d}p<0.0001$ significant increase as compared to diabetic control

modern therapy needs their pharmacological evaluation by modern methods. In the present study crude powder of *T. stocksianum* significantly reduced the blood glucose level both in normal and diabetic rabbits. The results obtained were in agreement with previous study (Akhtar et al., 2011). Hypoglycemic effect of different extracts: Ether, chloroform, ethyl acetate, methanol and aqueous extract of *T. stocksianum* were studied in diabetic rabbits. Ethyl acetate extract of *T. stocksianum* produced maximum decrease in blood glucose level which shows that the active principal(s) responsible for hypoglycemic activity is/are more extractable in ethyl acetate (Khushk et al., 2010). Ethyl acetate extract of *T. stocksianum* demonstrated synergistic effect with insulin which clearly shows that there may be some biological active principle(s) in ethyl acetate extract of *T. stocksianum* that exhibited insulin like action. The results were also in accordance with previous study (Maqsood et al., 2009). The extract significantly increased the serum insulin level in diabetic rabbits in one month study. The phytochemicals in the extract may produce direct insulinotropic

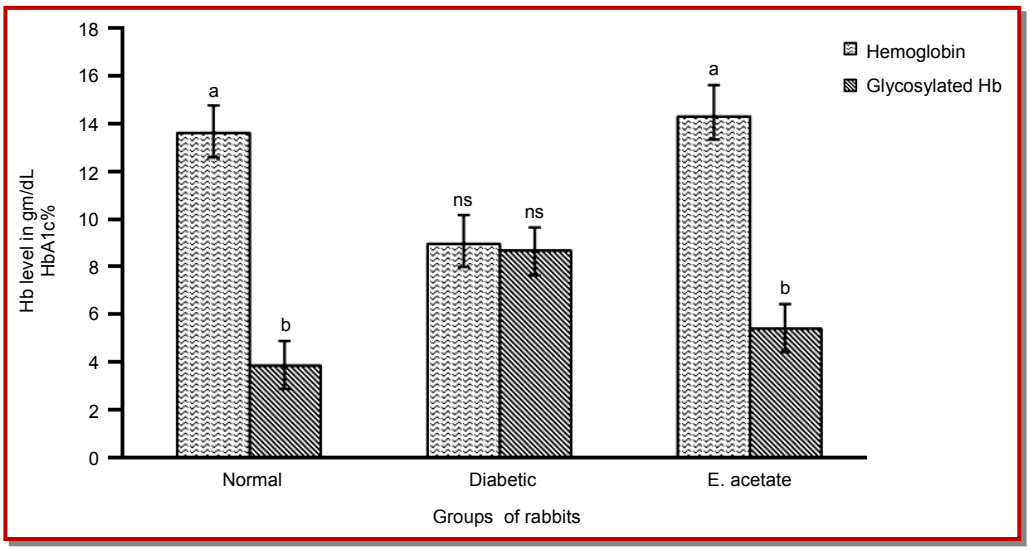

Figure 7: Effect of ethyl acetate extract of *Teucrium stocksianum* on hemoglobin (Hb) and glycosylated hemoglobin (HbA1c) levels in diabetic rabbits. (n = 6) where, ns = Non-significant change, [a]significant increase ($p<0.001$) [b]Significant decrease ($p<0.001$) compared to diabetic control as compared to diabetic control

effect and stimulate the release of insulin from the surviving β-cell in diabetic rabbits.

In oral glucose tolerance test, ethyl acetate extract inhibited the rise in blood glucose level after an oral glucose load in normal rabbits. Our finding is in line with the study of Tomar et al. (2012).

It has been reported that in diabetics, the total hemoglobin level is much lower than the normal level while HbA1c level is increased. Earlier reports show that during diabetes mellitus, excess of blood glucose reacts with hemoglobin to form HbA1c which is used as marker to determine the degree of protein glycation during diabetes and leads to the complications. Ethyl acetate extract significantly decreased the HbA1c level while increased the hemoglobin level in three month study. Earlier, it has been reported that aqueous extract of *Scoparia dulcis* decreased the HbA1c level thereby increasing the hemoglobin level in diabetic animals (Latha and Pari, 2004). The advanced glycated end products (AGE) are associated with severe complications of diabetes (McCance et al., 1992). So, ethyl acetate extract of *T. stocksianum* may be effective to reduce the diabetic complications.

It is concluded that ethyl acetate extract of *T. stocksianum* may contain active principle(s) that is/are responsible for its hypoglycemic effect. Further studies are required to isolate the active constituent(s) from the ethyl acetate extract of *T. stocksianum* and elucidate its exact hypoglycemic mechanism of action.

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