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PHARMACOLOGICAL EVALUATION OF ANTIDIABETIC EFFECT OF ETHYL ACETATE EXTRACT OF *TEUCRIUM STOCKSIANUM* BOISS IN ALLOXAN-INDUCED DIABETIC RABBITS

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ABSTRACT

Teucrium stocksianum Boiss has been used for the treatment of diabetes in folklore. Present study was designed to evaluate the effect of ethyl acetate extract of *Teucrium stocksianum* on blood glucose level in alloxan induced diabetic rabbits. The ethyl acetate extract produced significant ($P<0.001$) decrease in blood glucose level, ALT, AST, ALP, triglycerides, cholesterol, LDL, VLDL levels and T.C/HDL cholesterol ratio in diabetic treated rabbits as compared to diabetic control whereas, the HDL levels of diabetic treated rabbits were significantly ($P<0.001$) increased. It is concluded that ethyl acetate extract of *Teucrium stocksianum* possesses hypoglycaemic, hypolipidemic and hepatoprotective effects in alloxanized rabbits which strengthens its traditional use in diabetes.

Key words: Alloxan, hepatoprotection, hyperglycaemia, hypolipidemia, *Teucrium stocksianum.*

INTRODUCTION

The role of traditional medicines in the solution of health problems is valuable on a global level. World Health Organization has listed 21,000 plants which are used for medicinal purposes around the world (Modak *et al*., 2007). Plants based drugs are less expensive and are considered free from adverse effects. Various medicinal plants are traditionally used to manage diabetes and associated complications. However, there introduction into the modern therapy needs their scientific evaluation. *Teucrium stocksianum* Boiss (Labiatae), belonging to the family Labiatae is traditionally used for the management of diabetes and its complications on empirical basis (Ali and shah, 2011). The current study was planned to evaluate the hypoglycaemic activity of *Teucrium stocksianum* and its possible effects on lipid profile and liver enzymes in alloxan induced diabetic rabbits.

MATERIALS AND METHODS

Chemicals and drugs: Alloxan monohydrate was purchased from Sigma Chemicals Co. Glibenclamide was provided by Biorax Pharmaceuticals, Islamabad. For the determination of liver enzyme and lipid profile diagnostic kits (Merck Chemical Co., Germany) were used.

Biochemical analysis: Blood glucose level was determined by Optium Xceed Glucometer using glucose oxidized optium kits. Liver enzyme levels and lipid profile were estimated by using Microlab 300 (Merck Chemical Co., Germany). LDLs level was calculated by using following formula

LDL= total cholesterol – HDL – (triglyceride/5) **(**Fridewald *et al*., 1979**).**

Plant material used: Aerial parts of the plant were collected from the hills of Dherai 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. Dr. Jehandar Shah, Vice Chancellor Shaheed Benazeer Bhuto University Sheringle Dir (Upper). After collection, the unwanted parts and other adulterants 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 ºC in the refrigerator.

Preparation of Extract: Cold maceration method was used for the extraction of plant material. One kg of the dried powder was soaked in five litter of ethyl acetate for 48 hours with occasional shaking. It was passed through muslin cloth and then filtered through the filter paper. The extract was dried by using rotary evaporator.

Animal Used: Adult healthy rabbits of either sex of local strain (*Oryetolagus cuniculus*), weighing about 1.20-1.50 kg were used in the study. Animals were housed at standard conditions of temperature (23±12^{o}C), humidity (55±15%) and 12 h light (7.00-19.00). 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 that were used in accordance with the National Institute of Health (NIH) guide for the care and use of laboratory animals.

Induction of experimental diabetes: After an overnight fasting, rabbits were made diabetic by intravenous injection of fresh solution of 150mg/ kg body weight of Alloxan monohydrate (Akhter *et al*., 2002). Three days (72 hour) after injecting the alloxan-monohydrate, 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.

Experimental design: Rabbits were randomly divided into four groups of six animals each. Group 1 and 2 served as normal and diabetic control respectively and were administered orally 20 ml of 2% aqueous gum tragacanth solution. Group 3 received orally 600 µg/kg body weight of glibenclamide. Group 4 was administered 500 mg/kg body weight of ethyl acetate extract of *Teucrium stocksianum* by oral route (Ramesh *et al.*, 2006). Rabbits were treated for 30 days on daily basis. Blood glucose level, serum liver enzyme (ALT, AST, ALP) and lipid profile of all the rabbits in each group were estimated at 0 and 30th day.

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

RESULTS AND DISCUSSION

Blood glucose level: Ethyl acetate extract of *Teucrium stocksianum* significantly (P<0.001) reduced the blood glucose level of diabetic rabbits treated for 30 days. The results were comparable with glibenclamide which also significantly decreased the blood glucose level. There was no significant change observed in the blood glucose level of normal and diabetic control groups (Table1). Our results are in accordance with the study of Noor *et al.* (2008) who reported the anti-diabetic activity of *Aloe vera* in diabetic animals. Alloxan acts as a cytotoxic agent for beta-cells of islet of Langerhans and induces diabetes by causing cell necrosis (Jorns *et al*., 1997). The Reactive Oxygen Species mediates the cytotoxic action with increase in cytosolic calcium concentration, leading to rapid beta-cell destruction. These processes result into decreased insulin secretion and consequently elevated blood glucose level (Szkudelski, 2001). In the current study alloxan monohydrate administration to the rabbits resulted in hyperglycaemia which was reversed with the treatment of ethyl acetate extract of *Teucrium stocksianum* for a period of one month. The decrease in blood glucose level of diabetic rabbits by ethyl acetate extract of *Teucrium stocksianum* can be correlated with a number of possible mechanisms. The extract might exert its effect by preventing the death of β-cells and/or recovery of partially destroyed β-cells. Furthermore, the hypoglycemic action of the ethyl acetate extract might be due to its insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles. Antioxidant or free radical scavenging properties of the extract, in preventing these changes may also be considered to play a vital role. Similar study was reported by Burcelain *et al*. (1995) who demonstrated the possible anti-diabetic activity of herbal extract.

Table 1 Effect of ethyl acetate extract of *Teucrium stocksianum* on blood glucose level in diabetic rabbits at 0 and 30th day

Grouping of rabbits	Blood glucose level (mg/dl)	
	At 0 day	At 30th day
Normal Control	87.16±4.15	91.33±1.17^{ns}
Diabetic Control	275.83±4.07	277.66±3.31^{ns}
Ethyl acetate extract Treated	289.16±7.93	169.16±6.51^{c}
Glibenclamide Treated	279±4.84	151.5±3.07^{c}

Results are expressed as mean ± SEM (n=6) where, ^{ns} = Non-significant change and c= (P < 0.001) as compared to 0 day.

Liver enzymes: The ethyl acetate extract of *Teucrium stocksianum* significantly (P<0.001) reduced the three liver enzymes (SGOT, SGPT and ALP) levels from the start to the end of month. Glibenclamide also significantly reduced the liver enzymes however, significant increase in liver enzymes of the diabetic control group was observed. There was no significant change in liver enzymes of rabbits of normal control group (Table 2). Our results are broadly consistent with the previous study (Garba *et al.*, 2009). In the assessment of liver damage certain biomarkers viz. AST and ALT level are measured because liver damage arising from necrosis or membrane damage normally releases the enzymes into circulation; therefore, measurement of these enzymes in serum gives an indication for the health status of the liver (Schmidt and Schmidt, 1979). AST is abundantly found in mitochondria of hepatocytes. So, ALT is more specific to liver, and it is the most commonly used parameter for detecting liver injury. Serum ALP level is also used in assessment of liver functioning. The ALT, AST and ALP activity are frequently used as common biochemical markers to evaluate hepatic damage (Kozer *et al*., 2003).

The higher serum AST, ALT and ALP levels indicated that diabetes induces hepatocyte damage. ALT and AST are directly associated with the conversion of amino acid to keto acid and are increased in the diabetic condition. Begum and Shanmugasundaram (1978) have reported an increased level of AST and ALT in the liver of diabetic animals. In the present study elevated levels of

ALT, AST and ALP were significantly decreased by the oral administration of ethyl acetate extract for 30 days. It is suggested that protection of alloxan induced liver damage might be due to the result of free radical scavenging activity of the extract by intercepting the radicals involved in the hepatocyte injury or certain phytochemicals like flavonoids, triterpenoids and steroids present in the extract played an important role in hepatoprotection.

Table 2 Effect of ethyl acetate extract on ALT, AST and ALP in diabetic rabbits at 0 and 30th day

Liver Enzyme (IU/dl)	Normal control		Diabetic control		Glibenclamide treated		Ethyl acetate extract	
	At 0 day	At 30th Day	At 0 Day	At 30th Day	At 0 Day	At 30th Day	At 0 Day	At 30th Day
ALT	39.83±4.21	40.33±3.83^{ns}	83.0±2.22	159.50±12.11^{A}	79.16±10.64	41.50±3.77^{c}	87.66±11.9	43.66±3.66^{c}
AST	34.33±6.74	39.66±7.11^{ns}	65.00±5.82	90.16±12.39^{B}	71.00±2.47	55.66±7.89^{c}	64.83±7.22	46.00±1.84^{c}
ALP	261.33±6.19	256.66±11.63^{ns}	279±5.49	298.16±15.35^{B}	259±8.24	72.66±5.96^{c}	254.83±7.17	79. ±4.63^{c}

Results are expressed as mean ± SEM (n=6) ^{ns} = Non-significant change. A = (P<0.05), B = (P<0.01) and c = (P < 0.001) significant increase compared to 0 day.

Lipid profile: Ethyl acetate extract of *Teucrium stocksianum* significantly (P<0.001) reduced the triglycerides, cholesterol, LDL and VLDL levels in diabetic rabbits in one month study whereas the HDL levels of diabetic treated rabbits were significantly (P<0.001) increased. The T.C/HDL cholesterol ratio was significantly reduced in the treated group. Glibenclamide also significantly reduced triglycerides, cholesterol, LDL and VLDL levels while HDL levels of diabetic rabbits were significantly increased. Lipid profile of diabetic rabbits significantly increased from the start to the end of the study however, HDL level was significantly (P < 0.001) decreased. There was no significant change occurred in the lipid profile of the normal control group (Table 3). Our findings are in accordance with the previous study (Rajasekaran *et al*., 2005). An abnormal high concentration of serum lipids in diabetes is mainly due to increased mobilization of free fatty acid from peripheral depots which in turn is due to the activation of hormone sensitive lipase during insulin insufficiency. Dysfunctioning of lipoprotein lipase in insulin deficient state has also been reported to cause hypertriglyceridemia due to impaired catabolism of triglyceride-rich particles (Niemeije-Kanters *et al*., 2001). Hypertriglyceridemia is a common finding in diabetic patients and is responsible for vascular complications. Bruan and Severson (1992) studied that deficiency of lipoprotein lipase activity significantly contributes to the elevation of triglycerides in diabetes. After treatment with ethyl acetate extract of *Teucrium stocksianum*, there was a considerable restoration in HDL-c, TC, TG, VLDC and LDL-c levels to near normal level which shows that extract has hypolipidemic effect. Hence, it is evident that the plant extract may be helpful in controlling the metabolism of certain lipoproteins and may cause a significant attenuation in serum HDL and LDL towards normal levels.

It is concluded that ethyl acetate extract of *Teucrium stocksianum* has hypoglycaemic effect. Moreover, it is effective in diabetes associated with hepatic damage and dyslipidemias. Present investigation strengthens the folk use of *Teucrium stocksianum* in diabetes. However, further detailed studies are necessary to be carried out to isolate the active constituent (s) and elucidate the exact mechanism of action.

Table 3 Effect of ethyl acetate extract of *Teucrium stocksianum* on lipid profile in alloxan induced diabetic rabbits at 0 and 30th day

Lipid profile (mg/dl)	Normal control		Diabetic control		Glibenclamide		Ethyl acetate Extract	
	At 0 day	At 30th day	At 0 day	At 30th day	At 0 day	At 30th day	At 0 day	At 30th day
Triglyceride	100.16±4.9	86.83±6.5^{ns}	197.50±5.0	242.83±5.0^{A}	187.16±6.9	88.50±57.87^{c}	205.33±9.33	140.33±7.18^{c}
Cholesterol	25.66±8.35	26.5±9.330^{ns}	54.50±11.97	88.33±13.73^{A}	62.83±9.49	41.50±4.32^{c}	67.33±1.9	43.00±1.3^{c}
HDL	20.00±6.81	21.33±5.60^{ns}	20.66±4.45	10.50±4.41^{c}	19.33±1.86	25.66±2.16^{A}	17.66±2.94	24.50±1.87^{A}
LDL	4.40±1.58	4.53±1.34^{ns}	24.63±2.90	37.10±7.83^{A}	30.36±3.93	17.35±2.62^{c}	24.88±1.47	14.30±0.78^{c}
VLDL	17.36±2.42	19.33±2.81^{ns}	40.83±11.16	48.56±4.7^{B}	39.43±3.33	19.46±1.05^{c}	41.06±1.866	25.06±3.52^{c}
T.C/HDL	1.05±.31	1.84±.93	4.72±1.81	6.67±3.79^{A}	5.18±1.14	2.21±.37^{c}	6.86±2.74	2.48±.36^{c}

Results are expressed as mean ± SEM (n=6) where ^{ns} = Non-significant change. A = (P<0.05), B = (P<0.01) and c= (P < 0.001) significant increase compared to 0 day.

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