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Acute toxicity study and effect of *Teucrium stocksianum* boiss on blood glucose level and weight of glucose treated mice

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Abstract – *Teucrium stocksianum* boiss has been traditionally used in the treatment of diabetes mellitus. The current study was designed to evaluate the hypoglycemic activity of ethyl acetate extract of *Teucrium stocksianum* boiss in glucose-fed mice for 30 days. Oral administration of 500 mg/kg body weight of ethyl acetate extract significantly (P< 0.001) decreased the blood glucose level of 10% glucose-fed mice in comparison to control group. Ethyl acetate extract significantly increased body weight (P<0.05), heart (P<0.001), kidney (P<0.001), pancreas (P<0.05) and liver (P<0.01) weight in glucose-fed treated mice as compared to diabetic group. The median lethal dose (LD_{50}) was estimated through intraperitoneal (i.p) administration of the ethyl acetate extract in mice and was found to be 3250 mg/kg.

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Keywords – *Teucrium stocksianum* boiss, glucose, hyperglycemia, mice, acute toxicity

1. Introduction

Diabetes mellitus is a complex chronic disorder that disturbs the metabolism of carbohydrates, proteins and fat due to the lack of insulin secretion or reduced sensitivity of the tissue to insulin. Diabetes is usually associated with chronic high blood glucose concentration that could lead to morbidity and mortality [1]. The day by day increase in diabetic patient worldwide is likely to remain a significant threat to public health in the years to come. It is estimated that about 366 million people are likely to be diabetic by the year 2030 [2]. Several treatment options such as biguanides and sulfonylureas are presently marketed for the treatment of diabetes mellitus but none of these are safer in long run and mostly associated with unwanted side effects. Management of diabetes without any side effect is still a challenge to the health community [3]. Therefore, the interest in herbal medicine is increasing among the health professionals for the search of more safer and effective treatment [4]. Herbal medicine has a long history in the treatment of many diseases including diabetes mellitus. Many traditional medicines in use have been derived from medicinal plants. In the last few year, herbal medicine gaining popularity in the treatment of many diseases both in developing and developed countries due to their natural origin and safety. World Health Organizationhas listed 21,000 plants which are used for medicinal purposes around the world [5].

Teucrium stockaisnum belongs to the family Labiatae (Lamiaceae). Previous studies showed that *Teucrium stocksianum* boiss has antispasmodic [6], mucus protection [7], and hepato-protective activities [8]. Although, *Teucrium stocksianum* boiss has been traditionally used to treat diabetic patients while none of scientific data is available. Therefore, this study was aimed to determine the effect of *Teucrium stocksianum* on blood glucose level, body and internal organs weight and in glucose-fed mice for one month. Furthermore, acute toxicity of *Teucrium stocksianum* boiss was also determined.

2. Materials and methods

2.1. Equipments

Optium xceed glucometer (Abbott Laboratories, USA), rotary evaporator, electrical weighing balance, dissection box, desicator.

2.2. Chemicals and Drugs

Ethyl acetate, D-glucose and normal saline. Chemicals were purchased from Sigma Chemicals Co.

2.3. Collection, Identification and Preparation of Plant Extracts

Aerial parts of the plant were collected from the hills of Dherai Talash, Dir (lower) Khyber PhuktoonKhwa, Pakistan in April, 2010. The plant was identified and authenticated by taxonomist Prof. Dr. Jehandar Shah, Vice Chancellor Shaheed Benazeer Bhuto University Sheringle Dir (Upper) Pakistan. After collecting the plant, the unwanted part 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 placed in air tight container and stored at 4 ^{o}C in the refrigerator.

One kg of the dried powdered drug was extracted with ethyl acetate by cold maceration at room temperature for 24 h with occasional stirring and shaking. The macerate was filtered with several layer of muslin cloth and the extract was dried with the help of rotary evaporator below 40 ^{o}C. Dried extract was stored in the sealed container at 4^{o}C before use in experimental procedures [9][10].

2.4. Animals Used

Adult healthy mice of either sex, weighing 20-30 gm, were used in the study. They were obtained from the animal house of University of Sargodha, Sargodha. Animals were housed

at standard conditions of temperature (22 ± 3°C), humidity (55±15%) and 12 h lights (7.00-19.00) in the animal house of pharmacy department. The mice were fed on standard rodent chow and clean drinking water was provided ad libitum. The animal care and handling were conducted in compliance with National Regulation for Animal Research and approved by animal ethical committee of the university.

2.5. Acute Toxicity Study

Acute toxicity study was conducted according to the method described by According to this method adult healthy mice were divided into five groups of two animals each. Group I served as control and received normal saline (10 ml/kg) while other groups (Group 2, 3, 4 and 5) were given different doses of ethyl acetate extract in an ascending order that is, 1000, 1500, 2000, 2500 mg/kg body weight. All the doses were administered through intra-peritoneal (i.p) route. The mortality rate was observed for 24 hours. Since no mortality occurred so another five groups of mice were taken. One group served as the normal control while the other groups were treated with the various doses of crude extract in an increasing order that is, 3000, 3500, 4000 and 4500 mg/kg. The highest dose which did not kill any animal and the lowest dose which resulted in mortality were noted. LD_{50} was calculated by the geometric mean of these two doses [11].

2.6. Hypoglycemic Effect of Ethyl Acetate Extract of Teucrium stocksianum in Mice

It has been reported that the chronic administration of d-glucose in drinking water results in hyperglycemia [12]. The aim of this study was to evaluate the hypoglycemic effect of ethyl acetate extract of *Teucrium stocksianum* boiss in glucose-fed mice for one month. Adult healthy mice of either sex were divided into 3 groups of 10 mice each. Group 1 served as normal control and fed on standard mice feed with water ad libitum, Group 2 served as diabetic control and provided with 10% glucose solution in distilled water ad libitum for 30 days while Group 3 served as treated group and received orally 500 mg/kg body weight of ethyl acetate extract of *Teucrium stocksianum* along with 10% glucose solution. After 30 days, all the animals were anesthetized and sacrificed. Blood glucose level was estimated with the help of glucometer. Internal organs such as heart, kidneys, liver and pancreas were removed through abdominal surgery and each of the organs was weighed [13].

2.7. 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.

3. Results

3.1. Acute Toxicity

The acute toxicity study has shown that the LD_{50} of ethyl acetate extract is 3250 mg/kg body weight.

3.2. Hypoglycemic Effect of Teucrium Stocksianum Boiss

The The ethyl acetate extract of *Teucrium stocksianum* in a dose of 500 mg/kg body weight significantly ($P<0.001$) inhibit the blood glucose level to rise in glucose fed mice treated with ethyl acetate extract as compared to the untreated glucose fed mice (diabetic control) which were provided with only 10% glucose solution (Fig-1).

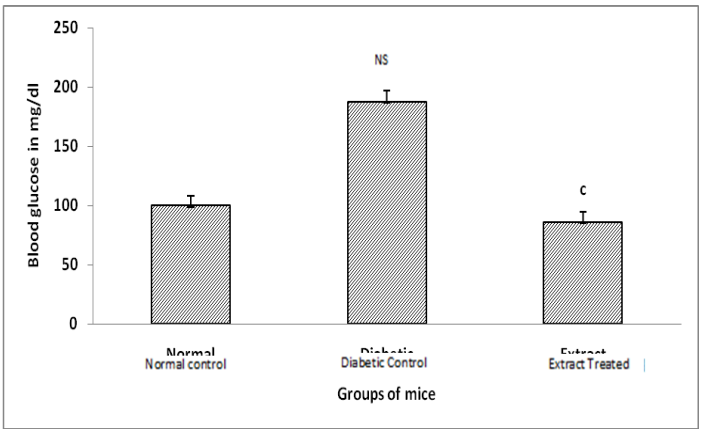

Figure 1. Effect of ethyl acetate extract of *Teucrium stocksianum* in Mice treated with 10% glucose

(n=10) where ^{NS} =Non-significant decrease, c=significant decrease ($P<0.001$) as compared to diabetic control.

3.3. Effect of Ethyl Acetate Extract of Teucrium Stocksianum Boiss on Weight of Mice

Ethyl acetate extract in a dose of 500 mg/kg body weight significantly increased the weight of the glucose-fed mice as compared to the mice treated with 10% glucose solution only (Fig-2).

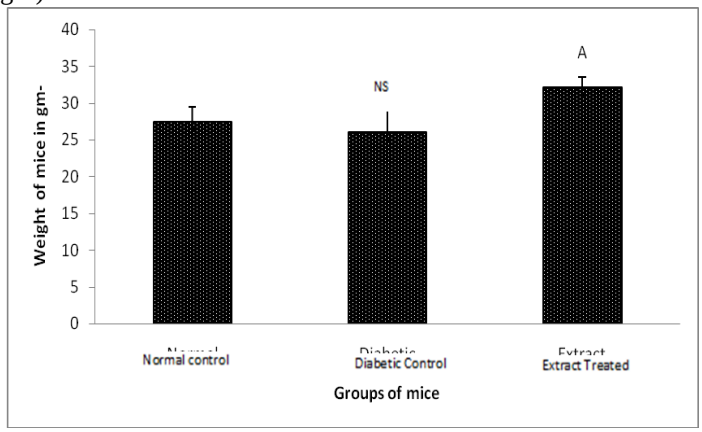

Figure 2. Effect of ethyl acetate extract of *Teucrium stocksianum* on weight of mice

(n=10) where ^{ns} =Non-significant change, A= significant increase ($P<0.05$) as compared to diabetic control.

3.4. Effect of Ethyl Acetate of Teucrium Stocksianum Boiss on Weight of Organs

Ethyl acetate extract produced a high significant ($P<0.001$) increase in the heart and liver weight of glucose induce diabetic mice as compared to diabetic control. However, significant ($P<0.05$) increase was noted in kidney and pancreas weight as compared to diabetic control (Fig-3)

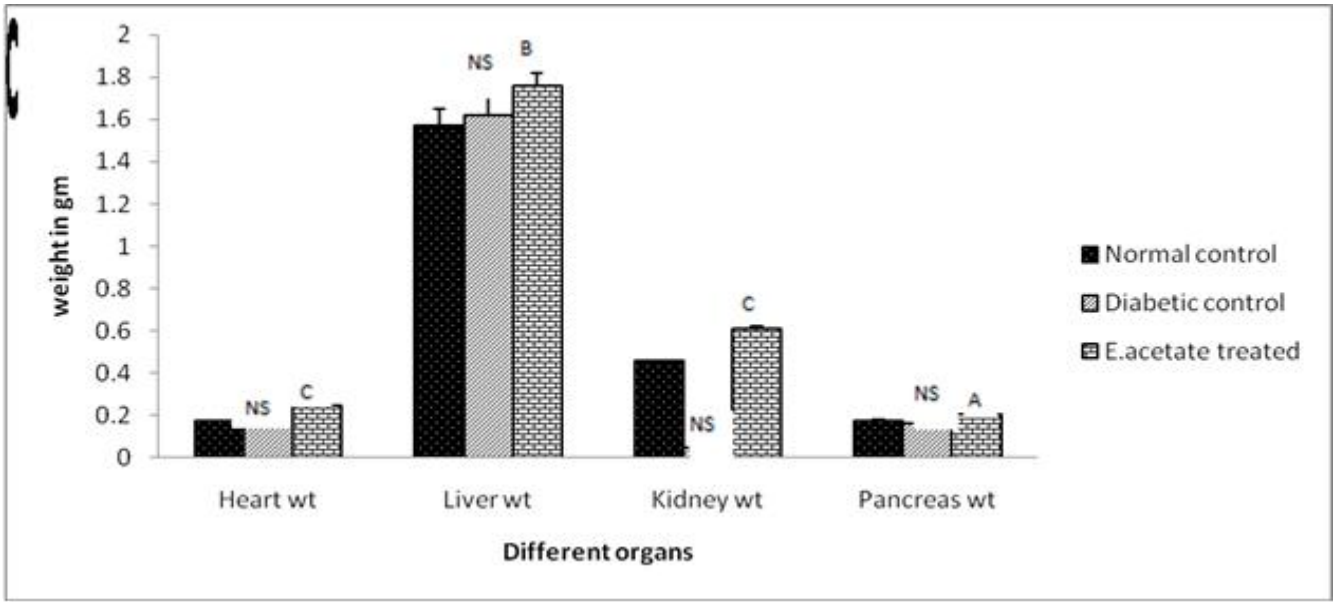

Figure 3. Effect of ethyl acetate extract of *Teucrium stocksianum* boiss on the weight of heart, liver, pancreas and kidneys of mice

(n=10) where ^{NS} =Non-significant change, A = (P <0.05), B= (P <0.01) and C= (P <0.001) significantly increase as compared to normal control (P<0.01).

4. Discussion

Herbal medicines have a long history in the treatment of diabetes and continue to be accepted currently as an alternative therapy. The scientific data have revealed that more than 1200 plants have found to possess anti-diabetic properties. Considerably large numbers of anti-diabetic plants are known through folklore however, pharmacological evaluation by scientific methods is required to establish the anti-diabetic activity. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future [14].

Midoui and Champlain, 2002, reported that chronic administration of high glucose diet resulted in non–insulin-dependent diabetes which is characterized by increased blood glucose level. The most important outcome of diabetes is the high blood glucose concentrations (hyperglycemia) [12]. Chronic exposure to high glucose concentration can produced toxicity and irreversible damage to the tissue which is a major cause of cellular dysfunction. This process is usually known as glucose toxicity. It has been reported that persistence and prolong high blood glucose lead to non-physiological and potentially irreversible β-cell damage [15].

The current study shows that chronic glucose feeding in mice for 30 days resulted in hyperglycemia which is in agreement with previous study [16]. The possible mechanism due to which the glucose fed mice developed diabetes may be due to the production of oxygen free radical (O_2^-) which damaged the pancreatic tissue as previously mentioned by [17]. Our results show that ethyl acetate extract of *Teucrium stocksianum* maintained the blood glucose level within normal range in chronic glucose-fed mice as compared to the control. The anti-hyperglycemic effect of ethyl acetate extract could be linked to number of possible mechanisms.

One of the mechanisms could be its possible stimulating effect on β-cell of pancreas to secrete insulin which is in accordance with previous findings [18] [19].

The anti-hyperglycemic activity of ethyl acetate extract of *Teucrium stocksianum* might be due to the presence of certain phytochemicals such as tannins, saponins, polyphenols and alkaloids and dietary fibers which have blood glucose lowering effect as studied earlier [20]. The current study correlates with the previous findings of Eseyin *et al*., 2002, who reported that the ethyl acetate extract of *Telfairia occidentalis* possesses phytochemical constituents which produce hypoglycemic effect [21]. However, another possible mechanism for antidiabetic activity of ethyl acetate extract may be due to the presence of micronutrients such as vanadium which has insulin-like action and lowered blood glucose level. It is reported that majority of plant extracts exert their blood sugar lowering effects via insulin-like micronutrients present in the extracts [22]. The improvement in insulin-stimulated glucose transport into muscle in treated mice might be responsible for the glucose lowering effect of ethyl acetate extract which was in line with previous study carried out by Friedman *et al*., 1992 [23].

Proteolysis, lipolysis and acute fluid loss during diabetes provided the basis for weight loss along with increased muscle wasting as studied by Alberti *et al*., 1989 [24] and Shirwaikar *et al.,* 2004 [25]. Persistence high blood glucose level is the main cause of tissue damage and weight loss in diabetic animals [15]. Ethyl acetate extract significantly (P<0.05) increased the body weight of glucose fed mice during 30 days study as compared to the diabetic control. The weight gain in treated mice may be due to the ability of the plant extract on proper tissue/cell utilization of glucose, therefore sparing the fat and reducing the rate of ketone body formation. The sparing effect on fat or reduction in fat oxidation therefore leads to the accumulation of fat and consequent increase in body weight. Another possibility may be due to controlling muscle wasting and improvement in insulin secretion as well as glycemic control by the extract.

Ethyl acetate extract produced a significant increase in

the weight of heart, kidney, liver and pancreas. However the macroscopic examination of these organs in treated group did not show significant change in colour and texture when compared with the normal control group. A number of possible mechanisms support this increase in the organ weights. The increase in the weight of these organs in ethyl acetate extract treated group may be due to presence of some phytochemicals in the extract that increase the functional ability of these organs and promote the health status of the vital organ which is supported by the previous study [26]. The increase in weight of these organs with the administration of ethyl acetate extract might be due to the regeneration of organ tissues that were damaged by the persistent high blood glucose level. The results are in agreement with Pandey,2010 [27], who studied antidiabetic and anti-hyperlipidimic effect of *Eugenia jambolana* seed and *Aegle marmelos* leaf extracts in alloxan induced diabetic rats. This further strengthens the hypoglycemic effect of the plant extract by the proliferation and regeneration of pancreatic tissue which is responsible for the secretion of insulin. The acute toxicity has shown that the ethyl acetate extract was found to be safe up to 3000 mg/kg which shows that the plant is not practically toxic and can be used without any toxic effect.

5. Conclusion

From this study it is cleared that ethyl acetate extract of *Teucrium stocksianum* boiss contains some active constituents which are responsible for maintenance of blood glucose level of treated group within normal range in glucose fed mice as compared to the diabetic control. So, it needs further attention and work to find out the constituents that are responsible for hypoglycemic activity and their exact mechanism of action.

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