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Studies on Antidiabetic Effects of Different Extracts from *Costus Speciosus (Koen)* Leaves

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Abstract: *Costus speciosus (Costaceae) commonly known as keukanda is a medicinal plant, used in Ayurveda for treating various diseases, one of which is diabetes mellitus. This work include study of Petroleum ether, Ethanol and Water (Aqueous) extract of powders of the fresh and dried leaves of Costus speciosus (CS) were organized and their blood glucose lowering effect compared by administrating them at the dose of 100 mg/kg and 200 mg/kg orally to diabetic rats. The ethanol powder extract of fresh leaves at a dose of 200 mg/kg body weight was found to significantly reduce fasting blood glucose, an effect comparable to that of glimepiride, a known synthetic drug. This extract was experienced for blood parameter study like SGOT, SGPT, Total cholesterol, Triglyceride, Total protein, Serum HDL cholesterol and alkaline phosphatase and also for glycosylated haemoglobion study. Thus the Costus speciosus an edible vegetable, appears to be a safe alternative to reducing blood glucose. The study was performing to explore antidiabetic potential as it is not reported earlier.*

Keywords: *Costus speciosus, Diabetes, SGOT, SGPT, HDL.*

Introduction

Diabetes is a disorder of carbohydrate, fat and protein metabolism attributed to diminished production of insulin or mounting resistance to its action. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. It is higher in developed than in developing countries. The number of adults with diabetes in the world will rise from135 million in 1995 to 300 million in the year 2025. [1]

Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries. [2] These may be delayed, lessened or prevented by maintaining blood glucose values close to normal. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues which regulate gastric emptying and inhibitors of intestinal alpha glucosidases like acarbose, miglitol and voglibiose which delay postprandial hyperglycemia. Sulphonylureas, the most widely used class of drugs act by closure of ATP dependent channel. Metformin, a biguanide oral antibiotic limits intestinal glucose absorption. These drugs have certain effects like causing hypoglycemia at higher doses, liver problems, and lactic acidosis and diarrhea. It is apparent that due to the side effects of the currently used drugs, there is a need for a safe agent with minimal adverse effects, which can be taken for long durations.

In addition to the above drugs of synthetic origin, many agents of plant origin are also in use particularly for the treatment of non-insulin dependent diabetes mellitus (NIDDM). A variety of medicinal plants like *Momordica charantia* L.,*Azardirachta indica* and *Ficus racemosa* are known to possess antihyperglycemic activity[3]. Extracts of the leaves of *Gymnema sylvestris* have been shown to induce beta cell regeneration.

A galactomannan has been identified as the major constituent of the blood glucose lowering extract from *Trigonellafoenum-graecum* [4].

For the present study *Costus speciosus* was chosen since it is by far the most extensively investigated and most widely acclaimed remedy for treatment of diabetes mellitus since ancient times. *Costus speciosus*, also referred to as keukanda, is a member of the Costaceae family and is commonly used as a traditional remedy for diabetes in India. In present work petroleum ether, Ethanol and water extracts of the fresh and dried leaves at the dose of 100 mg/kg and 200 mg/kg were compared for their efficacies in lowering fasting blood glucose. The ethanolic extract showed the maximum efficacy at the dose of 200 mg/kg.

PLANT PROFILE

Taxonomic Classification[5]

Kingdom	Plantae
Subkingdom	Tracheobinota
Super Division	Spermatophyta
Division	Mangoliophyta
Class	Liliopsida
Sub Class	Zingiberidae
Order	Zingiberales
Family	Costaceae
Genus	Costus
Species	Speciosus

Vernacular names

Assam	Tara
Bengali	Keu, Keumut
English	Spiral flag
Guajarati	Paskarmula, Valakdi
Hindi	Keu, Keukand, Kemuka, Kemua
Kannad	Changalvakostu, Chikke
Malayalam	Channakoova
Marathi	Penva, Pinnha, Kobee, Peva
Tamil	Kostam
Telegu	Kashmeeramu, Cengalvakostu
Sanskrit	Kembuka, Kebuka, Kembu

GEOGRAPHICAL SOURCE AND MORPHOLOGY

Geographical Source

Moist and wet evergreen areas of the Sri Lanka and Indo-Malyan within India,[6]*Costus speciosus* occurs throughout the foot hills of H.P, Assam, Vindhya and Satpura hills in Central India, Eastern Ghats of Andhra Pradesh and estern Ghats of Maharashtra, Karnataka, Tamil Nadu and Kerala[7]These plant species are also distributed in the Kalsubai- Harishchandragad wild life sanctuary, Ahmednagar[8]

Morphology

Ornamental, herbaceous plant, root stock tuberous stem, sub-woody at the base, thick creeping rhizomes (120-300 cm height) growing up to 2-2.7 m height with long lanceolate leaves and white fragrant flowers in terminal clusters.[9]

It is tall and dramatic landscape plant with large dark green, subsessile, elliptic or obovate leaves arranged on the stalk in spiral form. It can grow up to 3.1 m height in frost free areas but usually grows to about 1.8 m tall in cooler areas where its roots get harden but dies back in winter.[10] The plant flowers during the months of July and August, the aerial parts withering away during the winter season.The flowers look like crepe paper, thus the common name is"Crepeginger".The flowers are 5-6 cm long with a cup shaped labellum and crest yellow stamens. Fruit is red in color whereas seeds are black, five in number with a white fleshy aril. [11]

Traditional uses

This plant is used as food and medicine by the Kannikars, the primitive hill tribes of South India. Recently it is used in drug industry as a natural source of diosgenin which is a steroidal sapogenin used for synthesis of sex hormones, cortisone and oral contraceptives[7] Diosgenin content up to 3.37% has been reported in rhizome of *Costus speciosus*. The plant parts has many medicinal uses, juice of rhizome is applied to head for cooling and relief from head-ache, bruised leaves are applied in fever, decoction of stem is used in fever and dysentery, patients with high fever mostly utilize leaf infusion or decoction as a sudorific or in a bath, sap from leaves, young stems are used against diarrhea [5] cough, cuts, wounds, scabies, antidote for snake bite, jaundice, arthritis [12] burning sensation, constipation, leprosy, skin diseases, asthma, bronchitis, inflammations, anemia [13]worm infection, rash, nose pain, to stop vomiting used as antivermin and for abortion [14] The rhizomes of *Costus speciosus* are bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelminthic, depurative, febrifuge, expectorant, tonic improve digestion and is a stimulant herb that clears toxins.

The rhizomes have anti-fertility, anabolic properties. The rhizomes are generally consumed in the form of decoction. An alkaloid extract from rhizomes had papaverine like smooth muscle relaxant and enhances antispasmodic activities.

Rhizomes are also given in pneumonia, rheumatism, dropsy, urinary diseases, jaundice, and leaves are given in mental disorders. *Costus speciosus* rhizomes extract stimulate the uterine contraction due to non-estrogenic effects [15]. The plant is also used for eye and ear infections. Rhizomes have also been seen to exhibit cardiotonic, diuretic and CNS depressant activities, formerly used in Malaysia for small pox [16]. It is used as an ingredient in a cosmetic to be used on eyelashes to increase attractiveness. [17]

Phytochemistry

Diosgenin is the major constituent isolated from *Costus speciosus.* The maximum quantity of diosgenin reported in the stem is 0.65%, in the leaves 0.37% and in the flowers 1.21%. Other constituents isolated are Tigogenin, dioscin,gracillin-sitosterolglucoside. The seeds contain 6% of pale yellow sweet smelling fatty oil.[18] The fatty acid composition of the oil are- Palmitic- 55.97%; stearic- 8.3%; oleic-22.75%; linoleic- 6.8%; arachidic- 1.7%

Fig-1 *Costus speciosus* leaves

Table 1.Preliminary screening of *Costus speciosus*

Secondary metabolites	Name of the test	Inference
Alkaloids	Mayers test Dragendorffs	+ +
Flavanoids	Shinoda test Kellar Kiliani test	+ +
Cardiac Glycosides	Molisch test	+
Sterols	Libermann Burchard test	+
Tannins	Gelatin test	+

('**+**': present)

The physico-chemical properties

Specific gravity	0.9125
Refractive index	1.4672
Acid value	23.84
Saponification value	179.84
Iodine value	76.4

Methods and materials

Green, fresh leaves of *Costus specious* were collected from the Gondia district, and identified and authenticated (Voucher specimen no.2218 placed in herbarium of Bhavbhuti Mahavidyalaya Amgaon). Part of fresh leaves was shade dried to get *Costus speciosus* leaves dried powder. Streptozotocin were obtained from Sigma Co. Autoanalyser used AD-100 biochemistry analyzer, Glucometer, Hot air oven, Soxlets apparatus and digital microscope.

Experimental animals

S.D. rats weighing 150–200 g were issued from animal house the rats were housed in air-conditioned animal house. Each group was kept in a separate cage and fed pellet and boiled water ad libitum. The experiment performed by the permission of Animal Ethical Committee (IEAC) of P.Wadhwani College of Pharmacy Yavatmal (Wide no.650/PO/Re/S/2002/CPCSEA/2016/12).

Induction of diabetes in rats[19]

Diabetes was induced by single intraperitoneal injection of freshly prepared solution of Streptozotocin (STZ) at the dose of 50mg/kg in 0.1 Mcitrate buffer (pH 4.5) to the rats fasted overnight. After 3 days of STZ induction, FBG was checked and animals with abnormal FBG (110–250mg/dl) were treated as diabetic rats.

Experimental design of Protocol for Antidiabetic Activity

Group -I	Normal with daily dose of 0.5 ml of 0.5 % Tween 80 (vehicle) p.o
Group -II	Diabetic control with single dose of Streptozotocin (50 mg/kg.ip)
Group -III	Diabetic rats with daily dose of 0.8 mg/kg Glimepiride in 0.5% tween 80 p.o.
Group -IV	Diabetic rats with daily dose of 100 mg/kg Pet.Ether Extract in 0.5% tween 80 p.o.
Group -V	Diabetic rats with daily dose of 100 mg/kg Ethanol extract in 0.5% tween 80 p.o.
Group -VI	Diabetic rats with daily dose of 100 mg/kg Water extract in 0.5% tween 80 p.o.
Group-VII	Diabetic rats with daily dose of 200 mg/kg Pet.Ether Extract in 0.5% tween 80 p.o.
Group-VIII	Diabetic rats with daily dose of 200 mg/kg Ethanol extract in 0.5% tween 80 p.o.
Group -IX	Diabetic rats with daily dose of 200 mg/kg Water extract in 0.5% tween 80 p.o

Preparation of the extracts

Pet ether Extract (PEE)

This was prepared by extracting 0.5 kg of dried leaves of *Costus speciosus* with the Pet. Ether in a ratio of 1:10. The continuous extraction using soxlets apparatus was carried out at 50^0C. It was then filtered and evaporated to dryness under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder.

Ethanolic extract (EE)

This was prepared by extracting 0.5 kg of dried leaves of *Costus speciosus* using ethanolic extract in ratio of 1:10. The extraction was carried out exactly as described above. The yield was 39 g.

Water extract (WTE)

This was prepared by extracting 0.5 kg of dried leaves of *Costus speciosus* using water at a ratio of 10:25 by cold maceration method. It was then filtered and evaporated to dryness under reduced pressure. The yield was 19 g.

Mode of feeding

The rats were administered the materials twice a day for a period of 3 weeks. Different extract of leaves were orally fed at a dosage of 100 and 200 mg/kg body weight and glimepiride as a standard at a dosage of 0.8 mg/kg body weight. The rats were weighed everyday throughout the study. Fasting blood was collected from the tail vein once a week and glucose estimated. In the case of glimepiride and that extract which shows the anti-hyperglycemic activity, blood was collected from the animals by retro-orbital bleeding, at the end of the 3rd week, sera separated and used for the determination of biochemical parameters like SGOT,SGPT, Total cholesterol, HDL-cholesterol, , triglycerides, Total protein, Alkaline phosphates.

Statistical analysis

Statistical analysis was performed using Data analysed by Two-way ANOVA followed by Bonferroni post tests using statistics (for bodyweight and blood glucose level). The significance of difference between and within various groups was determined.

Values expressed as mean ± S.D., n=6

$^{\#}P < 0.05$ when compared with vehicle treated group.

$^{*}P < 0.05$ when compared with diabetic control group.

Results

a) Effect of *Costus speciosus* (100mg/kg) leaves extracts on body weight and blood glucose diabetic rat.

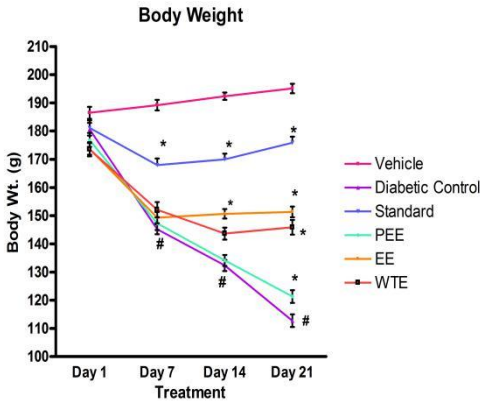

Fig 2: The effect of *Costus speciosus* (100 mg/kg) on body weight of rats.

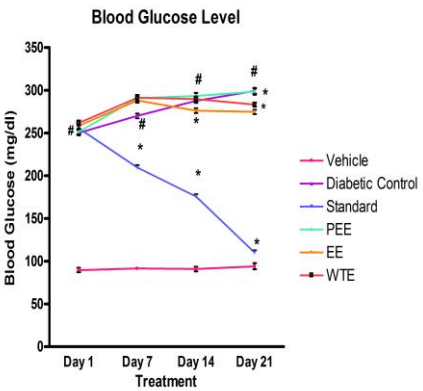

Fig 3: Effect of *Costus speciosus* (100mg/kg) extracts on blood glucose level of rat.

b) Three-week treatment with various extracts of Costus speciosus leaves (100 mg/kg) on blood parameter of diabetic rats (Day 21)

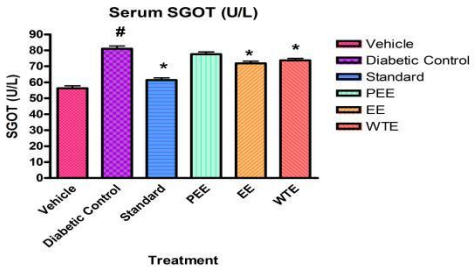

Fig.4: The effect of *Costus speciosus* (100 mg/kg) on SGOT of rats

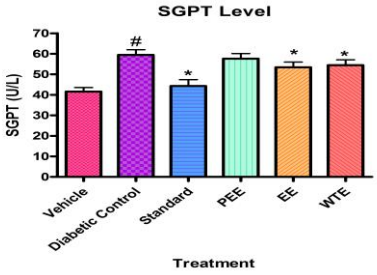

Fig.5: The effect of *Costus speciosus* (100 mg/kg) on SGPT of rats

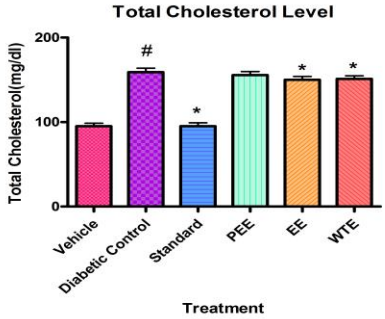

Fig.6: The effect of *Costus speciosus* (100 mg/kg) on Total cholestrol of rats

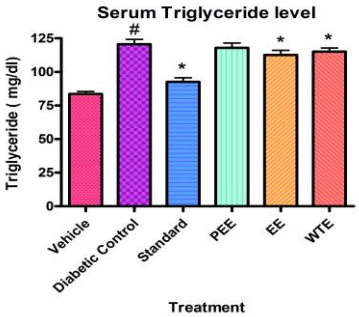

Fig.7: The effect of *Costus speciosus* (100 mg/kg) on Serum Triglycerides of rats

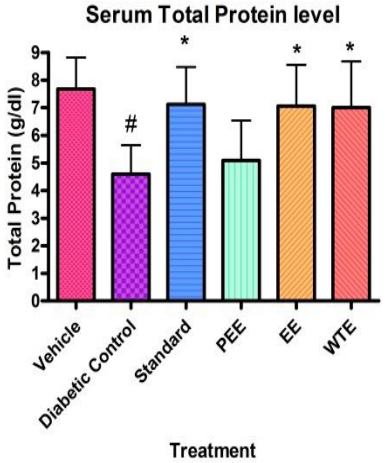

Fig.8: The effect of *Costus speciosus* (100 mg/kg) on Total protein of rats

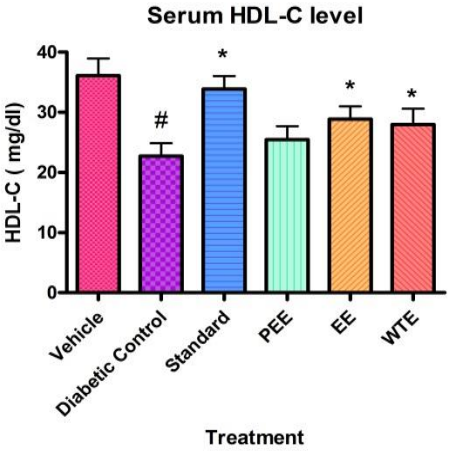

Fig.9: The effect of *Costus speciosus* (100 mg/kg) on HDL cholestrol of rats

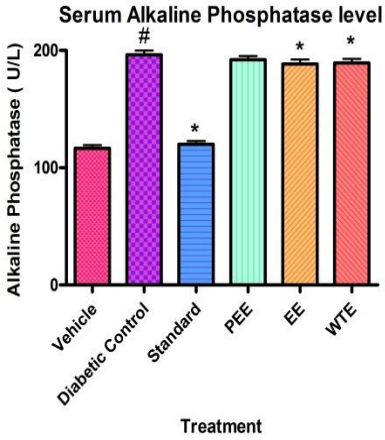

Fig.10: The effect of *Costus speciosus* (100 mg/kg) on Alkaline Phosphatase of rats.

c) Effect of *Costus speciosus* (200mg/kg) leaves extracts on body weight and blood glucose diabetic rat.

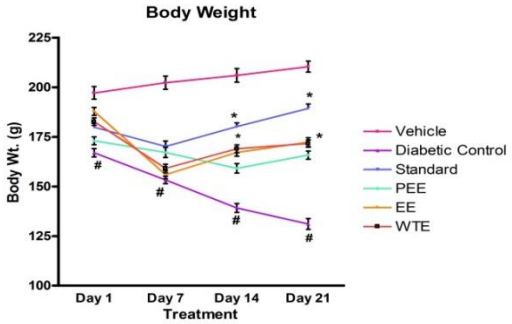

Fig 11: The effect of *Costus speciosus* (100 mg/kg) on body weight of rats

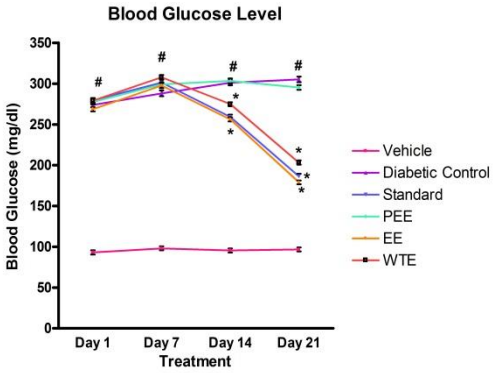

Fig 12: The effect of *Costus speciosus (100 mg/kg)* on blood glucose of rats

d) Three-week treatment with various extracts of Costus speciosus leaves (200 mg/kg) on blood parameter of diabetic rats (Day 21)

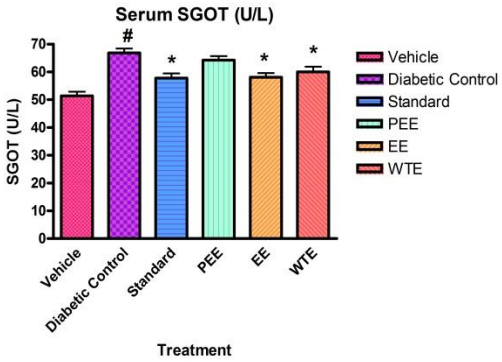

Fig 13: The effect of *Costus speciosus* (100 mg/kg) on SGOT of rats.

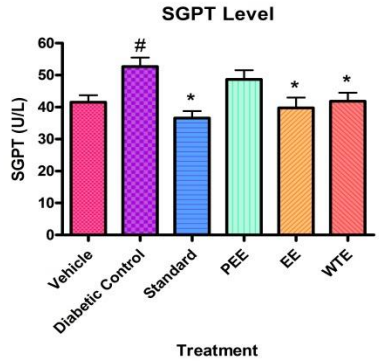

Fig 14: The effect of *Costus speciosus* (100 mg/kg) on SGPT of rats

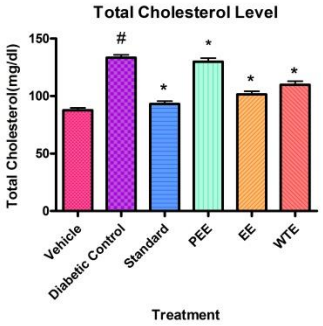

Fig 15: The effect of *Costus speciosus* (100 mg/kg) on Total cholestrol of rats

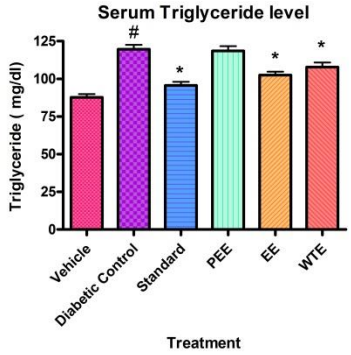

Fig 16: The effect of *Costus speciosus* (100 mg/kg) on Triglyceride of rats

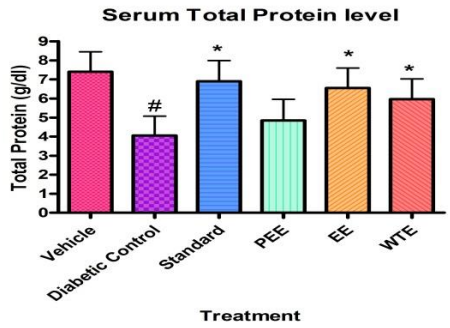

Fig 17: The effect of *Costus speciosus* (100 mg/kg) on Total Protein of rats.

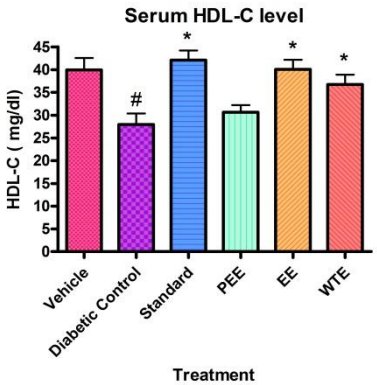

Fig 18: The effect of *Costus speciosus* (100 mg/kg) on HDL Cholestrol of rats

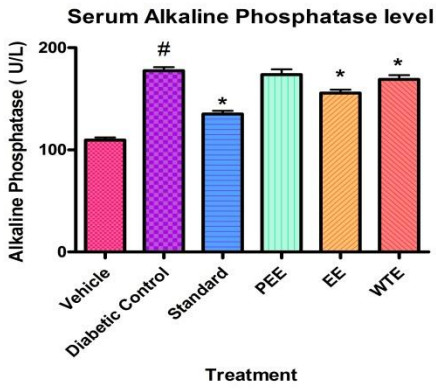

Fig 19: The effect of *Costus speciosus* (100 mg/kg) on Alkaline phosphatase of rats

e) Effect of *Costus speciosus* (200mg/kg) leaves extracts on diabetic rat.

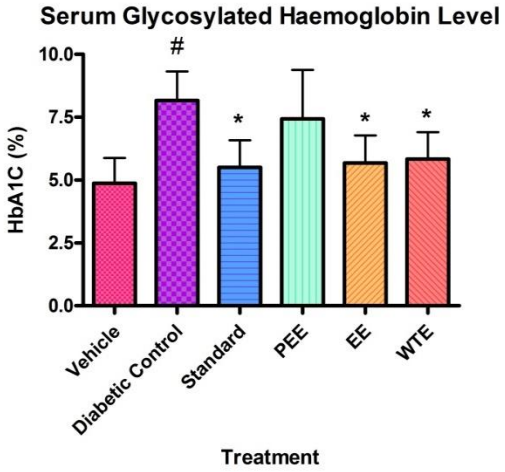

Fig 20 : Effect of Costus speciosus extract (200 mg/kg) on Glycosylated Hemoglobin(HbA1c)level of rats.

Results and discussion

As can see from graph, though all three extract powders did lower blood glucose, the ethanolic extract powder showed the maximum efficacy. The aqueous extract also lower blood glucose at the end of the first week and blood glucose lowering is also increase at the end of the third week. While the pet-ether extract showed almost no antihyperglycemic activity, the aqueous extract showed a blood glucose lowering activity, which was stayed consistent till the end of the study. The anti-hyperglycemic activity of extract was comparable to that of Glimeperide. The superior effects of the ethanolic extract from the fresh leaves is effective than aqueous extracts in lowering blood glucose. Ethanol was used for extract keeping in mind that certain active principles like phytosterols, can be extracted with ethanol. The oral feeding of extract ethanolic for 3 weeks resulted in an increase in body weight. The aqueous extract also lead to a gain in body weight being in diabetics and in controls. Pet ether extract doesn't show significant effect on body weight. The diabetic animals fed the extracts showed less weight gain than the corresponding controls. This observation and the decrease in body weight observed in uncontrolled diabetics might be the result of protein wasting due to unavailability of carbohydrate for utilization as an energy source. All the extract was chosen for a further detailed study of biochemical parameters. Glimeperide, a synthetic drug, was used as a reference for comparison. In this study, diabetes control rats exhibited significantly elevated SGOT, SGPT, cholesterol and triglyceride levels as compared to normal control rats. Treatment with ethanolic and aqueous extract significantly reduced SGOT, SGPT, cholesterol and triglyceride levels. It also improves total protein and serum HDL cholesterol. Maintenance of serum lipid profiles recommended the effectiveness of the extract against experimental type 2 diabetic rats.

In present study Glycosylated (or glycated) hemoglobin study was carried out using different extract of Costus *speciosus*. Glycosylated hemoglobin (Hb1c, or HbA1c, A1C) is a form of hemoglobin used primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. Glycosylation of hemoglobin has been implicated in nephropathy and retinopathy in diabetes mellitus. Monitoring the HbA1c in type-1 diabetic patients may improve treatment. The HbA1c level is proportional to average blood glucose concentration over the previous four weeks to three months and the result from the study indicate the improvement in HbA1c level after administration of plant extract[20].

I. Conclusion

A systematic study of three extracts from *Costus speciosus* leaves indicates that a ethanolic and aquous extract powder at 100 and 200 mg/kg body weight can reverse streptozotocin induced hyperglycemia in rats .

The extract ameliorated loss in body weight in diabetic animals. The advantage of this natural preparation lies in its ability not only to control hyperglycemia at low dosages but can also be taken for longer periods. Thus Costus speciosus, a widely consumed vegetable could be safely prescribed to

diabetic patients on a long term basis, which may also delay the onset of secondary Complications.

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