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Anti-Diabetic Activity of Aqueous extract of Cymbopogon flexuosus in high fat diet induced Obese Guinea pigs

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I. INTRODUCTION

The natural world has been a source of many medicinal plants for millennia, involving discovery with useful drugs from plant sources. Plants can be useful for structural modifications and optimization, as biochemical or pharmacological probes and as sources of inspiration for generations of synthetic organic medicinal source (Balandrin et al., 1993). Several pathogenic processes are involved in the development of diabetes. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissue (Tuomilehto et al., 2001). Insulin resistance caused by factors such as obesity and ageing is an important factor in case of diabetic state (Kahn 2003).

Cymbopogon flexuous; commonly known as lemongrass and locally called Cochin or Malabar grass is indigenous to India and grown in Kerala, Assam, Maharashtra and Uttar Pradesh (Kumar, Shukla et al. 2009). C. flexuous leaves accumulate substantial amounts of essential oils and its five components are Citral, Eugenol, geraniol, alpha pinene and linalool (Sangwan, Singh-Sangwan et al. 1993). The anti-inflammatory and analgesic activity of Cymbopogon flexuosus was studied (Chandrashekar and Prasanna). The anti-cancer activity of essential oils was obtained from C. flexuosus (Sharma, Mondhe et al. 2009). Cymbopogon is one such plant which is yet to be investigated in regard to its anti-diabetic property along with other pharmacological activity. Therefore the present study was carried out to determine anti-diabetic activity on aqueous extract of C. flexuous in high fat diet induced Obese Guinea pigs.

II. EXPERIMENTAL DESIGN

Preliminary photochemical screening

Phytochemical evaluation comprises of different chemical test and chemical assay. The concentrated plant extracts were subjected to preliminary screening for the detection of various plant constituents presents.

Test for Alkaloid: Mayer's test, Wagner's test, Hager's test, Dragendorff's test were performed.

***α* –amylase Inhibitory activity:**

Selection of Animal Species

Guinea pigs weighing (250-300gm) were used in the experiment. Animals were maintained under uniform laboratory conditions (12h light and 12 hour dark cycle,25±30ºC) in standard polypropylene cages and provided food and water ad libitum.The animal study was permitted by the Institutional animal Ethics Committee and bearing IAEC No.**HPI/2018/60/IAEC/PP-0162.**

Acute toxicity study

Healthy young mice were employed for performing this acute toxicity study. Each animals at the commencement of the dose were 6 weeks old. Total 21 animals were selected for studying this activity. In this study the different doses of extract (20mg, 40mg, 80mg, 160g, 320mg, 640mg and 1280 mg/kg body weight) of the doses were considered. The extract was dissolved in water and the volume of liquid that can be administered should not exceed 1ml/100g of body weight.

Procedure:

Administration of doses: Animals were fasted for 18 hours prior to administration of the test substances. Following the period of fasting, the animals were weighed and the test substance was administered. The test substance at different doses were administered by gavage using a stomach tube. 20mg/kg was considered as starting dose.

Microscopic examination of organs showing evidence of gross pathology in animals surviving 24 or more hours were considered. At the end of the experiments, all animals were sacrificed for histomorphological study. After sacrificing the parts of the liver, heart, kidney and brain were collected and washed with normal saline. The

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tissue were immediately fixed in 10% formalin for a period of 24 hour, dehydrated with alcohol, embedded in paraffin, cut into 4-5µm thick sections, and attained with haematoxylin-eosin dye for photo microscopic observation. The microscopic features of organ of mice were compared with the control group.

Preparation of high fat diet

The High fat diet was prepared according to the formulation stated by (Muthu, Sethupathy et al. 2005)

S.No.	Ingredients	Quantity (%)	Quantity(gms)
1	Carbohydrate (Wheat Flour)	40	400
2	Protein (Soyabean Bori)	15	150
3	Polysaccharide (Sucrose)	03	30
4	Fat (Vegetable oil) +Animal fat (Lard oil)	36	360
5	Salt mix (Nacl: Kcl:$Cacl_2$)(5:1:2)	04	40
6	Fibres (Cellulose)	01	10
7	Vitamin (Verimol)	01	10

Table No 1: Composition of high fat diet

High fat diet induced diabetes

High fat diet was administered for diabetes induction. Animals were divided into 5 groups consisting of 6 animals in each. Parameter like Fasting blood glucose, body weight were estimated on the day of initiation and on 5th, 10th, 15th and 20th day during the study.

Treatment schedule:

Group 1: Normal control (vehicle)
Group 2: High fat diet group (High fat diet)
Group 3: High fat diet + Test extract (30mg/kg)
Group 4: High fat diet+ Test extract (90mg/kg)
Group 5: High fat diet+ metformin (500mg/kg)

Blood was withdrawn by following cardiac puncture at the end of study i.e. on 20th day. Once the blood was withdrawn it was then centrifuged at 3000rpm for 15mins for collecting serum. Serum was thus collected and used for estimating blood glucose level estimation by using glucometer (Accu-Check).

Statistical analysis

All statistical analyses were performed using PRISM software. All values were presented as means ± S.D. (standard deviation). Comparisons among groups were made by application of two-way analysis of variance ANOVA followed by Bonferroni test. Differences were considered statistically significant if $p<0.05$, $p<0.01$ and $p<0.001$.

III. RESULTS

Acute toxicity study (LD50) of C. flexuosus extract:

Aqueous extract of C.flexuosus at different doses (20, 40, 80, 160, 320, 640, and 1280 mg/kg) was considered for acute toxicity study. Initial doses of 20 mg/kg had no adverse effect on the behavioural responses of the tested mice upto 14 days of observation. Physical observation indicated no sign of changes in the skin, eyes mucus membrane, behaviour patterns, salivation, and diarrhoea of the mice. However, the higher dose of the extract i.e. 1280 mg/kgafter the the gross examination at autopsy and histopathological evaluations revealed the leukocyte aggregation which are primary factor released during inflammation.

Therefore, this study indicates that C.flexuosus extract at higher dose can cause for acute toxicity effect. However, 30mg/kg and 90 mg/kg were considered for further study.

Histopathological Study of aqueous Extract of Cymbopogon flexuosus

After sacrificing the mice, parts of the liver, kidney, brain and heart were collected for histological study. The acute toxicity (LD50) study

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did not result in any mortality of treated mice. However, gross necroscopy of the different organs when observed under the light microscope using multiple magnification and at higher doses of extract showed a toxicity.

TREATMENT	MEAN BODY WEIGHT CHANGE(gms)			
	0 day	10th day	15th day	20th day
Normal control (normal diet)	292±8.3	290±8.7	291±8.9	293±9.0
Diabetic control (vehicle)	290±8.1^{ns}	315±8.3*	350±8.7***	371±9.2***
C.flexuosus (30mg/kg)	291±8.1***	289±8.0*	284±7.9***	274±7.2***
C.flexuosus (90mg/kg)	287±8***	284±7.9***	279±7.3***	277±7.1***
Metformin (500 mg/kg)	298±8.3***	287±8.0***	284±7.9***	272±7.0***

Values are expressed as means ±SEM ;(n=5).*P<0.05, **P<0.01, ***P<0.001 when Diabetic control group compared with Normal
control and P<0.05 when Diabetic control group compared with treated group.

Table No: 2 Mean change in body weight (gms)

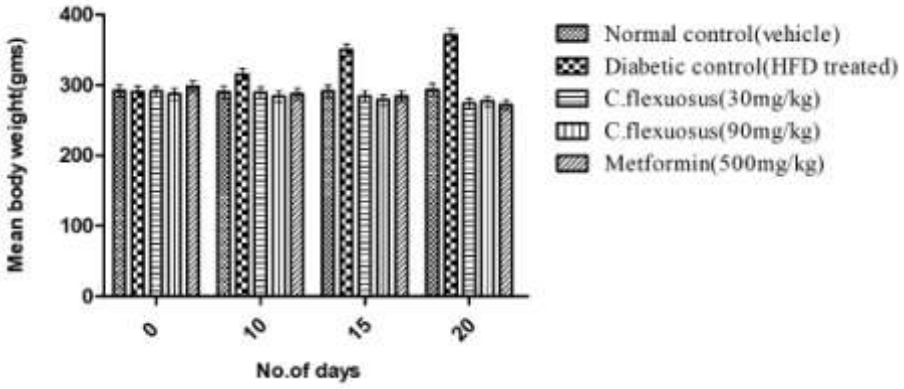

Fig:3 Effect of C.flexuosus on body weight (gms) of guinea pig at different days.

TREATMENT	MEAN FASTING BLOOD GLUCOSE (mg/dl)				
	0 day	5th day	10th day	15th day	20th day
Normal control (normal diet)	65±5.4	66±5.9	63±5.2	64±5.4	67±6.1
Diabetic control (vehicle)	110±4***	107±7.8***	108±6.2***	101±6.3***	104±5.5***
C.flexuosus (30mg/kg)	121±6.4	108±9.4^{ns}	88±12.4**	70±5.1***	68±5.1***
C.flexuosus (90mg/kg)	117±4.1	89±7.8**	81±13.5***	76±11.5***	69±6.4***

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Metformin (500 mg/kg)		111±5	85±6.5***	83±7.3**	73±9.8***	63±6.9***

Values are expressed as means ±SEM ;(n=5).*P<0.05, **P<0.01, ***P<0.001 when Diabetic control group compared with Normal control and P<0.05 when Diabetic control group compared with treated group.

Table No: 4 Mean fasting blood glucose level (mg/dl)

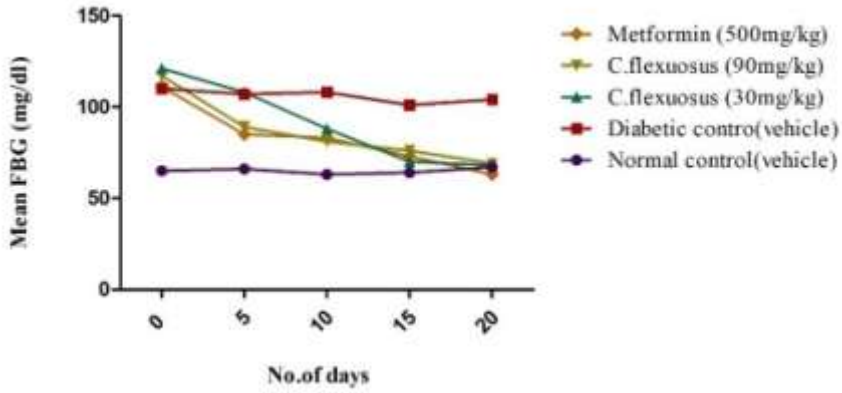

Fig: 5 Effect of C.flexuosus On Fasting Blood Glucose level (mg/dl).

α –amylase inhibitory activity of C.flexuosus:

Among the extracts different concentration, C.flexuosus strongly inhibited alpha amylase. All test were performed in triplicate. It showed IC50 for C.flexuosus was found to be 16.8±0.06% respectively.

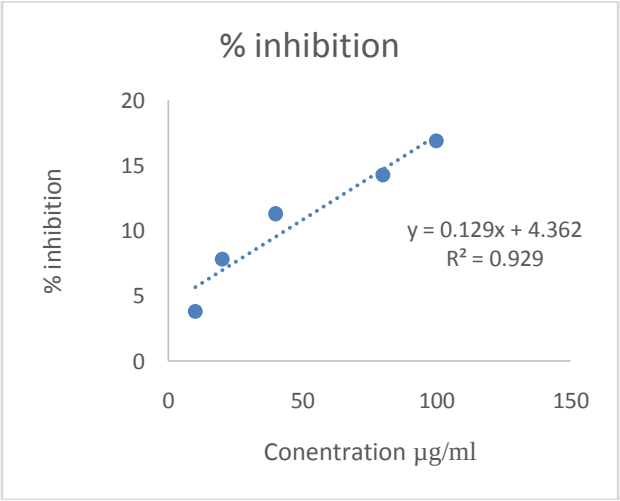

Fig:1 Effect of different conc.of C.flexuosus on alpha amylase inhibition**.**

Concentration(µg/ml)	10	20	40	80	100
% inhibition	3.6±0.13	7.6±0.13	11.4±0.11	14.4±0.13	16.8±0.06

Values are expressed as means ±SEM ;(n=5).*P<0.05, **P<0.01,***P<0.001

Table No:7 % inhibition of C.flexuosus at different concentration.

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TNF alpha level of the study

Twenty days treatment of the study animal with C.flexuosus (30mg/kg) significantly lowered the TNF alpha level.The C.flexuosus**(**90mg/kg) group showed a (0.19±0.009) as compared to the C.flexuosus(30mg/kg) and showed a much better effect in reducing the TNF alpha level. All the test were performed in triplicate.

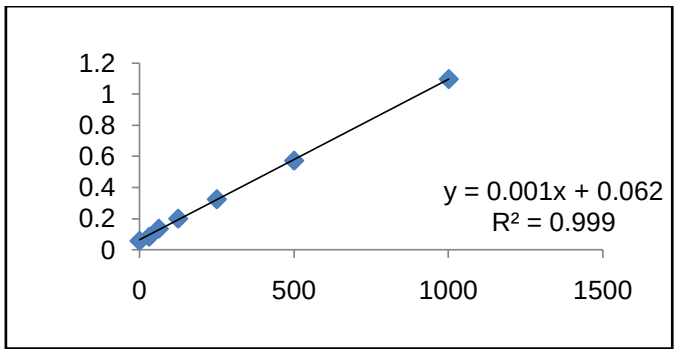

Table No 6 TNF alpha level of the study animals

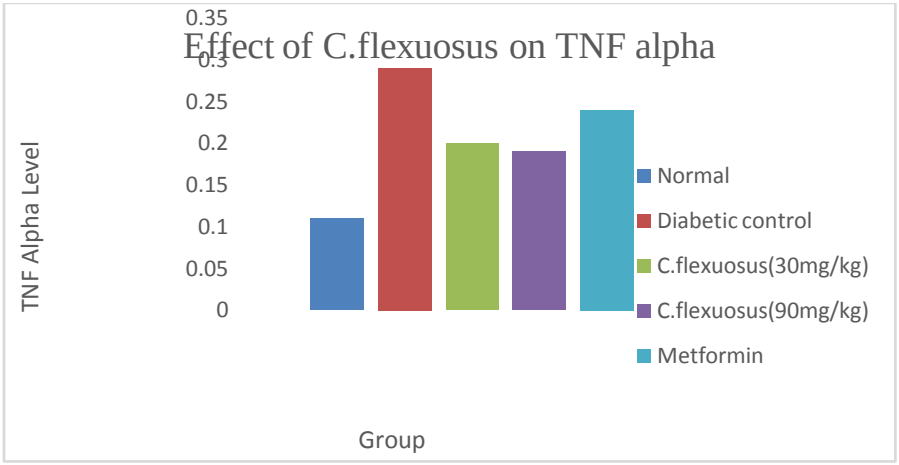

Fig: Level of TNF alpha after treatment with C.flexuosus.

IV. DISCUSSION:

Diabetes mellitus is one of the common metabolic disorder and 1.3% of the population suffers from this disease throughout the world. Although the genetic and environmental factors are major cause of obesity, modern eating habits, especially consuming HFDS have been identified as the most important reason for increased risk of diabetes (Matsuzawa-Nagata, Takamura et al. 2008).

In our present study, we found that serum blood glucose was significantly decreased in both the groups (30mg/kg C.flexuosus and 90mg/kg C.flexuosus treated). It could be due to additive effect shown by C.flexuosus by inhibiting alpha amylase and another by reducing release of TNF alpha.

Prolong inflammation is always associated with HFD induced obesity. An important early studies on the interplay among obesity, inflammation and insulin resistance showed that TNF alpha expression was elevated in adipose tissue and showed that immune neutralization of TNF alpha in obese fatty rat ameliorate insulin resistance The insulin resistance type 2 diabetic guinea pigs when treated with C.flexuosus different doses showed to reduce the TNF alpha level. Reduction in the level of TNF alpha may be due to the administration of C.flexuosus. The actual mechanism by which C.flexuosus extract is able to reduce the level of blood glucose is not known but the decrease in blood glucose may be due to mechanism shown by inhibiting alpha amylase and reducing TNF alpha level which is confirmed by Elisa test of TNF alpha. An important early studies on the interplay among obesity, inflammation and insulin resistance showed that TNF-α expression was elevated in adipose tissue.

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Amylase inhibitors are also known as starch blockers because they prevent dietary starch from being absorbed by the body and thereby lower postprandial glucose levels. Slowing the digestion and breakdown of starch may have beneficial effects on insulin resistance and glycaemic index control in people with diabetes (Uddin N. et al., 2014).

In our investigation we found that Aqueous leaves extract of C.flexuosus moderately inhibited α-amylase and showed IC50 value (16.8±0.06) µg/ml.

Therefore, this study buttress the claim that natural inhibitors from dietary plants have α-amylase inhibitory activity and could be used as effective therapy for the management of postprandial hyperglycaemia with minimal side effects.

V. CONCLUSION:

The aqueous extract of Cymbopoon flexuosus were subjected for preliminary phytochemical investigation and it showed the presence of alkaloids. Acute toxicity study (LD50) of the aqueous extract of the Cymbopogon flexuosus was performed which implied 1280 mg/kg b.w. as the cut off dose. Therefore, 30 and 90 mg/kg were selected for screening anti-diabetic activity respectively.However, the extract was shown to have cellular dmage when given at higher doses. Concominent treatment with aqueous extract at the dose of 30 and 90 mg/kg showed market reduction in blood glucose whereas, the high fat diet group showed a marked increase in blood glucose level. The alpha amylase inhibitory activity of the plant also concludes that the extract at concentration of 100 µg/ml showed IC50 value as 16.8±0.06%.

In summary, the aqueous leaves extract of Cymbopogon flexuosus have been found to exert a beneficial action in high fat diet induced diabetes either by inhibiting alpha amylase or by reducing release of TNF alpha. It is concluded that this herb might have a potential to be further developed into an anti-diabetic phytomedicine.

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