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In-vitro study of anti-diabetic and anti-obesity using α-amylase inhibitory of herbal extracts fenugreek, green coffee beans, cumin seed and Ajwain.

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Abstract:

The aspect of medicines supplied to patients. clinch that the medicines prescribed to patients are suitable. For the purpose of Safety and Efficacy of medicines it should be benefit for a patient and drug ability to produce the optimum results. Secretion of insulin which result hyperglycemia comes under the group of Diabetes mellitus, insulin action, or both and obesity is a chronic metabolic disorder caused by an imbalance between energy intake and expenditure. The present work is about anti-diabetic as well as anti-obesity drug, which is formulated using extract of fenugreek, green coffee beans, cumin seed and trachyspermum ammi (ajwain) by using in-vitro α-amylase inhibitory activities of plant extracts. The purpose of this study is to evaluate the α-amylase inhibitory activity of different plant extracts of against porcine pancreatic amylase in vitro. Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. The regulation of fatty acid and triglyceride availability in biological responses depends on the activity of lipolytic enzymes present in fatty acid metabolism in adipose tissue. This project is about antidiabetic as well as antiobesity drug, which is made with the using Fenugreek (methi) is widely used for antidiabetic as well as obesity control, Green Coffee Beans (coffee Arabica) which is used in weight loss, lowers the blood pressure, Cumin Seed (Bunium bulbocastanum) which is used as diabetes, gastrointestinal disturbances and also helpful in weight loss and Trachyspermum ammi

(Ajwain) it also work for diabetes.This is helpful for the people those who are suffering from diabetes and as well as obesity.

Keywords: Plant extract, Antidiabetic and anti-obesity activity, Trachyspermum ammi, in-vitro α-amylase inhibitory activities, Cumin Seed.

1. Introduction:

Diabetes mellitus, commonly known as diabetes type II, is one of the world's oldest known diseases.[1] WHO estimated in 1995 that the number of people with diabetes in the world would reach 300 million by 2025. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[2] The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal genitourinary and cardiovascular symptoms and sexual dysfunction. [3] Since Diabetic mellitus is a multifactorial disease, the available pharmaceuticals, despite their sensible treatment, target mostly one pathway to control hyperglycemia and encounter several side effects. Traditionally, antidiabetic plants and /or their active constituents may fulfill this need. [4] Diabetes mellitus is an chronic metabolic disorder. In this body is unable to make proper use glucose that causes high blood sugar. Obesity is a global health problem. It is also known to be a risk factor for the development of metabolic disorders, type 2 diabetes, systemic hypertension, cardiovascular disease, dyslipidemia, and atherosclerosis. [5] This eventually increases the urine output which leads to dehydration and increases thirst. Compared with synthetic drugs, drugs derived from the plants are frequently considered to be less toxic with fewer side effects. [6] Therefore, the search for the more effective and safer antihyperglycemic agents becomes an area of active research. in the development of diabetes. [7] The extracts, powder and gum of fenugreek seeds and leaves have been reported to have anti-diabetic and hypocholesterolemic properties in both model animals and human.Obesity is a global health problem. It is also known to be a risk factor for the development of metabolic disorders, type 2 diabetes, systemic hypertension, cardiovascular disease, dyslipidemia, and atherosclerosis. In this study, we screened crude extracts from 400 plants to test their anti-obesity activity using porcine pancreatic lipase assay (PPL; triacylglycerol lipase, EC 3.1.1.3) *in vitro* activity.[8] Among the 400 plants species examined, 44 extracts from plants, showed high anti-lipase activity using 2,4-dinitrophenylbutyrate as a substrate in porcine pancreatic lipase assay. [9] Furthermore, 44 plant extracts were investigated for their inhibition of lipid accumulation in 3T3-L1 cells. Among these 44 extracts examined, crude extracts from 4 natural plant species were active. [10] These results suggest that four potent plant extracts might be of therapeutic interest with respect to the treatment of obesity.

2. Materials and methods

2.1. Materials

All herbal drugs purchased from market then the ingredients were triturated well and were passed from sieve no 30 to get the granules of uniform shape and size. All the chemicals and reagents used in this study were of analytical grade. 2.5L of ethanol (95%) using Soxhlet extractor for 2 days. After the extraction period, solutions were filtered and concentrated by rotary vacuum evaporator. Extract was subjected to different chemical tests for the detection of various phytoconstituents. [21]

- Fenugreek (methi) which is the main constituent of the drug along with other contains galactomannan as an essential constituent, which lowers the digestion and absorption of carbohydrates, thus it helps to lower the blood glucose levels.
- The green coffee bean from the drug helps in weight loss by the constituent Chlorogenic acid.
- Ajwain is used as flavouring agent as well as in losing appetite as it contains thymol and gamma terpines.
- Therefore, the altogether drug will help in three ways: 1) Antidiabetic
  2) Weight loss
  3) Loss of appetite (obesity).

Table 1-Details of herbal drugs used for study of antidiabetic as well as obesity [22]

S.N.	Drug Name	Synonym	Family	Chemical constituent	Uses
1	Fenugreek [25]	Trigonellafoenum-graecum	Fabaceae	Galactomannan	1) Used as antidiabetic agent 2) Obesity reducer 3) Used in production of milk in mothers 4) Soothe skin inflammatory or injury
2	Green coffee bean [23]	coffee Arabica	Rubiaceae	Chlorogenic acid	1) Used in weight loss 2) lowers the blood pressure 3) antioxidant 4)used as antidiabetic agent
3	Ajwain [24]	Trachyspermum ammi	Apiaceae	thymol and gamma terpines	1) used in indigestion 2) recovering wounds 3) may be used in weight loss 4)used as antidiabetic agent
4	Cumin Seed	Black cumin or Bunium bulbocastanum	Apiaceae	Cumin aldehyde	1) Used as antidiabetic agent 2) Gastrointestinal disturbances 3) Helpful in weight loss

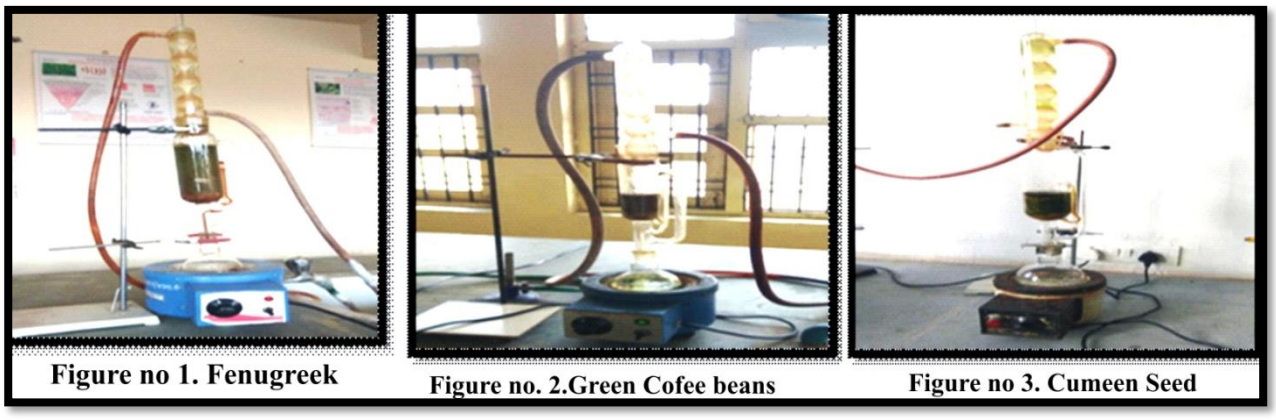

Figure no 1. Fenugreek

Figure no. 2.Green Cofee beans

Figure no 3. Cumeen Seed

2.2. Preparation of extracts

The plants were extracted three times with ethanol and chloroformand extracts were obtained through the removal of the solvent during evaporation. Each extract was evaporated using rotary evaporator, under reduced pressure. Different concentrations (0.1 µg/mL, 02 µg/mL, 03 µg/mL and 0.4µg/mL) of each extract were made by using dimethyl sulfoxide (DMSO) and subjected to α-amylase inhibitory assay using starch as a substrate. The concentrated samples were stored at −20 °C for further study.Using this method, the percentage of α-amylase inhibitory activity was calculated. Extracts were dissolved in DMSO at a final concentration that did not affect enzyme activity within the total volume (1%).

2.3. Alpha-amylase inhibitory activity of plant extracts

The α-amylase inhibitory activity of the extract was carried out according to the standard method with minor modification. [26] Ten microliter of porcine pancreatic amylase solution (0.1 mg/mL) were mixed with 30 µL of plant extracts or phosphate buffer (the control), or positive control (acarbose, 60µg/mL) and pre-incubated at 37 °C for 10 minutes. Then, 40 µL starch solution was added to initiate reaction and incubation was done at 37 °C for 30 minutes, then 20 µL of 1 M HCl and 75 µL iodine reagent were added to the 96-well plate.This was done to exclude false positive results, as some plants extracts have been reported to contain traces of α-amylase or starch. [27]

3. Result and Discussion

Within the context of present study, antidiabetic activity and antiobesity activity of extractswas investigated by using in vitro assay techniques.

3.1. α-amylase inhibitory activity

The plant extracts were prepared sequentially with ethanol and chloroform.[28] Each extract was evaporated using rotary evaporator, under reduced pressure. Different concentrations (0.1 µl, 0.2 µl, 0.3 µl, and 0.4 µl,) of each extract were made by using dimethyl sulfoxide (DMSO) and subjected to α-amylase inhibitory assay using starch as a substrate.[29] Colour comparative study was done for α-amylase inhibitory assay. α-amylase inhibitory assay performed by using such concentration in Table 2

Table 2- α-amylase inhibitory assay performed by using such concentration.

Concentration	0.1µl	0.2 µl	0.3 µl	0.4 µl
Control	0	0	0	0
Fenugreek	20	22	24	24
Green Coffee Beans	15	17.5	17	18
Cumin Seed	15	15	17	18
Trachyspermum ammi	20	20.5	21	22
mixture of drug	70	77.9	88.3	97.5

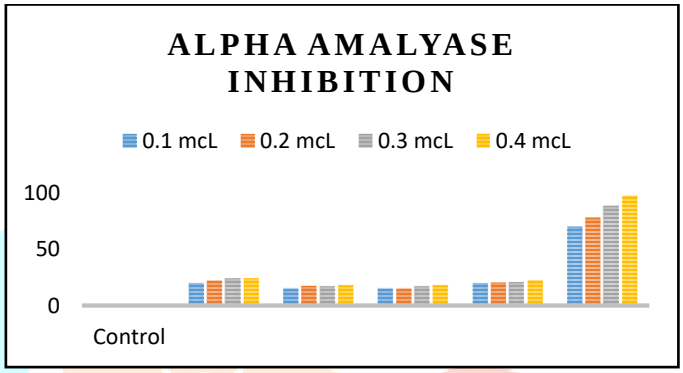

Figure no. 4 α-amylase inhibitory

Although, the percentage inhibition of the positive control (acarbose) was 94.91 ± 1.77% at 0.4 µg/mL, it ranged from 21.87 ± 0.31% to 39.67 ± 2.19% and 13.99 ± 1.05% to 20. 00± 0.73% for the chloroform and ethanol extracts, respectively. [30] Using this method, the percentage of α-amylase inhibitory activity and IC_{50}values of each extract was calculated. The IC_{50} values are depicted in table 3.

Table 3- IC_{50} values for α-amylase

Name of Extract	IC_{50} values (µg/mL)
Chloroform	396.46 ±1.87
Ethanol	757.69±3.57

Data are presented as values of mean ± SD. Mean separation by LSD ($p < 0.05$). Set of bars (the same concentration). Lower than acarbose (positive control). SD: Standard deviation; LSD: Least Significant Difference.α-amylase inhibitory activity was performed forChloroform and ethanol extracts.

4. Conclusion

Data obtained from this study suggest that both the aqueous and ethanol extract of fenugreek, green coffee beans, cumin seed and trachyspermum ammi exerts mild inhibitory effect on α-amylase. Further, the results revealed that both extracts could be a good candidate for antidiabetic and anti-obesity evaluations in vitro study. In addition, these results support the traditional use offenugreek, green coffee beans, cumin seed and trachyspermum ammiin the management of obesity. This study supports the ayurvedic concept that ethanol and chloroformof plant extracts exhibit considerable α-amylase inhibitory activities. This study also supports to usage in ethnomedicines for management of diabetes.Present study reveals that all plant extract has beneficial effects on blood glucose level as well obesity. To the best of our knowledge, these plant extracts have not been previously screened for their lipid inhibitory activity.Common advantages of herbal drugs are effectiveness, safety, and acceptability.

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