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Antidiabetic potential of Guizotia Abyssinica cass seed oil in Wistar Albino rats - An experimental approach

Research Article

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Abstract

Guizotia abyssinica Cass. widely grown plant of southern India, seed oil of which are said to be healthy source of oil. Diabetes mellitus is a metabolic disorder affecting major population worldwide. Nutritive healthy source of oils and fats are essential in current scenario. Hence antidiabetic property of *Guizotia abyssinica* Cass seed oil is tested in Streptozotocin induced diabetes in Wistar albino Rats. Materials and methods: Test drug collected, authenticated, seed oil prepared. Study was conducted in Wistar albino Rats inducing diabetes with Intra peritoneal injection of Streptozotocin. The animals under different test groups received Glibenclamide as standard drug; single and Double dose of *G. abyssinica* Cass seed oil as test drug and the effect was observed by analysing serum glucose, lipid profile, weight of the Liver and histopathological changes in liver and pancreas after 21 days of experiment. Results: Test drug disclosed decrease in the Serum Sugar level. It has also shown decrease in total cholesterol, LDL and not showed the significant increase in HDL. Histopathological observation of Liver and pancreas proved safety aspect of the drug by preventing the cytotoxic effect of STZ.

Key Words: Quercetin, Chrysin, Diclofenac, Tramadol, Hyperalgesia, Analgesics, Analgesiometer.

Introduction

Diabetes Mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms like life style, food habits, lack of exercises and many (1). The long-term hyperglycaemia usually leads to serious damage to many of the body systems, especially the nerves, blood vessels, heart, eyes, and kidneys, and thus concurs serious macro vascular and micro angiopathy complications, including retinopathy, nephropathy, and peripheral neuropathy (2). Latest data from International Diabetes Federation (IDF) and World Health Organization (WHO) indicate that DM now affects a staggering 246 million people worldwide, with 46% of all those affected in the 40- 59 age group during their economically most productive years, and this is likely to increase to at least 380 million by 2025 (3).

Many of the herbal plant sources mediate their anti-diabetic potential through mitigating oxidative stress (OS), promoting insulin secretion, few by inhibiting gluconeogenesis and glycogenolysis, thereby regulating blood glucose. The multifactorial pathogenicity of DM demands a multimodal therapeutic approach. Future therapeutic strategies might require the combination of various types of antidiabetic agents (4), to meet the demand of pathology at various stages.

Guizotia abyssinica Cass widely grown plant of southern India, yielding seeds, which are good source of edible oil (5). These are commonest household sources for preparing food articles like chutney, condiments etc (6). The seeds are reported to contain nutritional components like oil 30-40%, proteins 10-25%, and soluble sugars 12-18%, crude fibre 10-20%. The (fixed) oil is a mixture of triglycerides, lauric, palmitic, palmitoleic, stearic, oleic, linoliec and arachidic acids (7). The unsaponifiable matter is said to be mixture of stigmasterol n-triacontane and lupeol (8). Though popularly used food source less studies have been conducted on this.

Hence with all this background Experimental study has been designed to evaluate antidiabetic activity profile of *G. abyssinica* Cass. through Streptozotocin induced diabetes in Wistar albino Rats.

Materials and methods

Drug source

Matured seeds of *Guizotia abyssinica* Cass were collected from *Gadag* district, cleaned properly from extraneous matter, authenticated using floras and botanist's opinion. The oil was extracted by cold compression method through pressing the seeds in motorized Ghani. The extracted oil was decanted, filtrated, stored in air tight bottles and sample deposited at SDM centre for Research in Ayurveda and Allied sciences.

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Selection of Animals

Healthy Albino rats not less than 8 weeks old of either sex, weighing about 150 -200g were selected from animal house of SDM Centre for Research in Ayurveda and Allied sciences, Udupi. The experimental protocol was approved by IAEC with approval no. SDMCRA/IAEC/ DG-08. The rats were fed with normal diet, water throughout the study. They were acclimatized in the laboratory condition for two weeks prior to the experiment. The housing provided had the controlled lighting of 12:12hr light and dark cycle, 25ºC temperature and approximately 50% of relative humidity (9).

Study design

Antidiabetic study on STZ induced on Wistar albino Rats (10).

Posology

The human dose was decided by traditional survey on human consumption pattern and the same was converted into animals as per body surface area (PAGET AND BARNES formula 1964) (11)

Traditional dose for test drug *G. abyssinica* Cass seed oil is 48ml

Rat dose = Human Dose × 0.018×5 = 0.43ml/kg

Experimental Protocol

Hyperglycaemia was induced by a single intra-peritoneal injection of a freshly prepared solution of Streptozotocin (STZ) 40 mg/kg body weight in 0.1M cold citrate buffer of pH 4.5(12). The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycaemia. One-week later glucose range of 250mg/dl above was considered as hyperglycaemia. These animals were taken for the experimental study.

Grouping

Total 30 healthy albino rats were selected and grouped as shown in below table.

Table no 1: Grouping of Wistar Albino rats for experimental study

Sl. No	Group	No	Drugs	Dose
1	**Normal Control**	6	Normal diet and Water 0.5% CMC solution	10ml/kg
2	**Positive control**	6	Inj. Streptozotocin intra peritoneal as single dose	40 mg/kg
3	**Standard**	6	Inj. Streptozotocin intra peritoneal as single dose + Glibenclamide with 0.5% CMC solution	10mg/kg
4	**TED (Single Dose Test group –I)**	6	Inj. Streptozotocin intra peritoneal as single dose + *G. abyssinica* Cass. seed oil (Single Dose)	0.43ml/kg
5	**TED X2 (Double Dose Test group – II)**	6	Inj. Streptozotocin intra peritoneal as single dose + *G. abyssinica* Cass. seed oil (Double Dose)	0.86ml/kg

Animals of all groups provided free access to food & water throughout the study period. Standard drug & trial drug were administered to animals of the respective groups individually as per standard conversion rate of dosage. Test drug was administrated orally in morning hours for 21 days. On 22nd day, after overnight fasting, blood was collected from retro-orbital puncture and sent for biochemical investigation, then rats were sacrificed with ether overdose. The liver and Pancreas were excised out from sacrificed animal, weighed, and transferred in 10% formalin solution for tissue fixation prior to histopathological examination.

Weight of liver, histopathological study of Liver and Pancreas were taken as observational parameters. Blood sugar, Serum total cholesterol, Serum triglyceride, HDL cholesterol, LDL cholesterol, VLDL cholesterol were among biochemical parameters for the study.

Statistical analysis

The data obtained were analysed through ANOVA, F test, followed by Dunnett's t-test using as the post hoc test (Graph Pad 9.200). P value <0.05 were considered as statistically significant. The data were presented in table as Mean ± SEM and Graphs (13).

The percentage decrease in the Serum Sugar and lipid lowering was calculated using the following formula

$$\text{Sugar or lipid Lowering (\%)} = \frac{\text{Positive control Sugar or Lipid value} - \text{Sugar or Lipid value of STD or test drug}}{\text{Positive control Sugar or Lipid value}} X\ 100$$

Results

Effect on Serum Sugar

Table 2: Comparative effect on Serum sugar (mg/dL)

Groups	Mean ± SEM	% Change
Control	93.00 ± 2.03	-
Positive Control	548.2 ± 33.04***	489.46 ↑@
Standard	316.5 ± 36.93***	42.26 ↓ #
TED	349.5 ± 30.32***	36.25 ↓#
TEDX2	514.8 ± 17.84	6.09 ↓#

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05, **p<0.001

Effect on Serum Cholesterol

Table 3: Comparative effect on Serum Cholesterol (mg/dL)

Groups	Mean ± SEM	% Change
Control	70.33 ± 4.73	-
Positive Control	48.33 ± 3.84**	31.28↓@
Standard	75.33 ± 6.06***	55.86 ↑ #
TED	46.50 ± 3.74 (@)	3.79 ↓#
TEDX2	71.83 ± 3.38**	63.46 ↑ #

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05, **p<0.001

Effect on Serum Triglycerides

Table 4: Comparative effect on Serum Triglycerides (mg/dL)

Groups	Mean ± SEM	% Change
Control	89.67 ± 5.22	-
Positive Control	93.50 ± 3.43	4.27 ↑@
Standard	87.67 ± 17.82	6.24 ↓ #
TED	39.00 ± 4.48*	58.29↓#
TEDX2	152.83 ± 20.41**	63.46 ↑#

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05,**p<0.001

Effect on Serum HDL

Table no 5. Comparative effect on serum HDL (mg/dL)

Groups	Mean ± SEM	% Change
Control	45.00 ± 9.43	-
Positive Control	38.67 ± 3.90	14.07 ↓@
Standard	30.00 ± 4.06	22.41 ↓ #
TED	17.00 ± 0.52*	56.03 ↓#
TEDX2	41.17 ± 4.54	6.46↑ #

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05,**p<0.001

Effect on Serum LDL

Table no 6. Comparative effect on Serum LDL (mg/dL)

Groups	Mean ± SEM	% Change
Control	20.38 ± 0.70*	-
Positive Control	22.97 ± 2.23	12.71↑@
Standard	18.28 ± 0.53*	20.42↓ #
TED	17.20 ± 0.87 *	25.12↓#
TEDX2	35.33 ±1.74	53.81↑#

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05,**p<0.001

Effect on Serum VLDL

Table no 7. Comparative effect on Serum VLDL (mg/dL)

Groups	Mean ± SEM	% Change
Control	17.93 ± 1.04	-
Positive Control	18.70 ±0.69	4.29 ↑@
Standard	17.53 ± 3.56	6.24↓ #
TED	7.80 ± 0.90*	58.29↓#
TEDX2	30.57 ± 4.08**	63.46 ↑#

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05,**p<0.001

Effect on Weight of Liver

Table no 8. Comparative effect on Weight of Liver (mg)

Groups	Mean ± SEM	% Change
Control	6.47 ±0.30	-
Positive Control	10.23 ±0.38***	58.11↑@
Standard	7.44 ± 0.26**	27.27↓ #
TED	7.32± 0.12**	28.44↓#
TEDX2	7.98 ± 0.38 **	21.99↓#

Data: Mean ± SEM, @-Compared with Normal Control, #-Compared with Positive Control,*p<0.05, **p<0.001

Discussion

Guizotia abyssinica Cass seeds, widely used as one of the food components and as source of edible oil said to be healthy and best in diabetes. Diabetes Mellitus is a spectrum of common metabolic disorders, arising from a variety of pathogenic mechanisms, resulting in hyperglycaemia (14). This present paper designed to provide the data related to Antidiabetic activity of these seeds in Streptozotocin induced diabetes in Wistar albino rats. The animals under different test groups received Glibenclamide as standard drug; single and Double dose of *G. abyssinica* Cass seed oil as test drug and the effect was observed by analysing serum glucose, lipid profile, weight of the Liver and histopathological changes in liver and pancreas after 21 days of experiment.

Table no. 9. Overall antidiabetic property of *Guizotia abyssinica* Cass seed oil in Streptozotocin induced diabetes in Wistar albino Rats.

Sr. No	Parameters	Positive control*	*Guizotia abyssinica* Cass Seed oil Single Dose**	*Guizotia abyssinica* Cass Seed oil Double Dose **
1	Serum Sugar	SI	SD	NSD
2	Serum Cholesterol	SD	NSD	SI
3	Serum Triglycerides	NSI	SD	SI
4	Serum HDL	NSD	SD	SI
5	Serum LDL	NSI	NSD	NSI
6	Serum VLDL	NSI	SD	SI
7	Liver weight	SI	SD	SD

*Changes with reference to normal control rats,

** Changes with reference to Positive control rats. NSI-No significant increase, NSD- No significant decrease, SI- Significant increase, SD-Significant decrease

Serum Sugar

In present study, there was a significant elevation (489.46%) in blood glucose level in positive control group as compared with normal animals. The test drug treated group exhibited significant reduction (36.25%) of serum glucose levels as compared to the positive control group with $p \leq 0.001$. Double dose test drug showed mild decrease (6.09%) in sugar level. Over production of glucose by means of excessive hepatic glycogenolysis and gluconeogenesis is one of the fundamental bases of hyperglycaemia in diabetes mellitus (15).

Lipid profile

Most observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. As exceptional in present study significant decrease in serum cholesterol (31.28%) was observed but mild increase in triglycerides (4.27%), LDL (12.71%) and VLDL (4.29%) and decrease in HDL (14.07%) cholesterol have been observed in positive control rats.

Insulin deficiency results in failure to activate lipoprotein lipase thereby causing hypertriglyceridemia (16). There was a significant control of the levels of serum lipids in test drug treated diabetic rats. Serum cholesterol showed the mild decrease in single dose test drug group whereas the marked elevation (63.46%) in double dose test drug group.

In diabetes, LDL carries cholesterol to the peripheral tissues where it is deposited, whereas HDL transports cholesterol from peripheral tissues to the liver and thus aids its excretion. Hence increase in LDL is atherogenic. In present study, there was a significant decrease in TG(58.29%), LDL(25.12%), VLDL(58.29%) in single dose test drug treated group whereas double dose test drug treated does not showed the significant effect in TG(63.46%), LDL(53.81%), VLDL (63.46%). The HDL showed the significant decrease (56.03%) in single dose and significant increase (6.46%) in double dose test drug group.

The weight of liver showed significant increase due the cytotoxic effect, in positive control group (58.11%). The weight of Liver has significant decrease showing the protective action of test drug in single dose (28.44%) and double dose (21.99%).

Figure 1. Histopathology of Liver

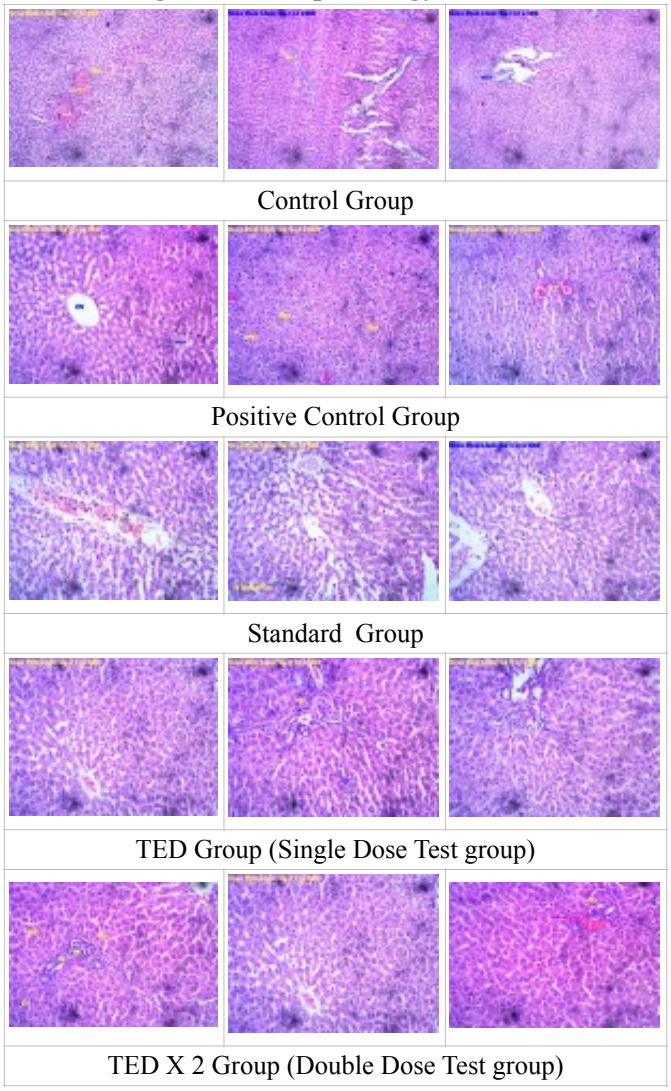
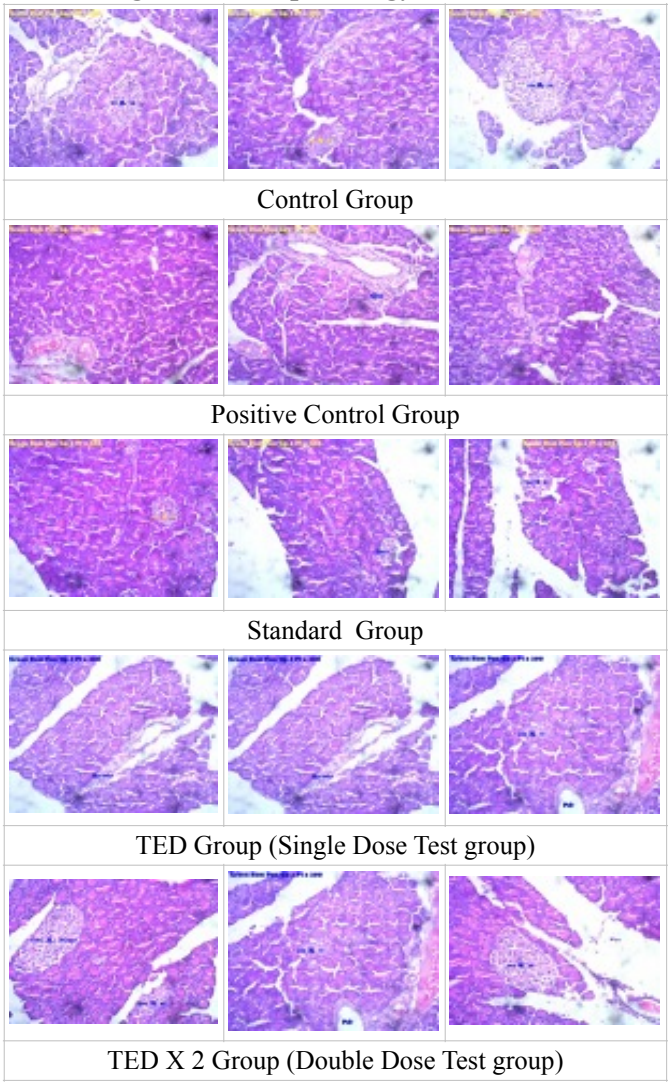


Figure 2. Histopathology of Pancreas



Histopathological change

Liver

Injection of STZ resulted in mild fatty changes, sinusoidal dilatation, diffused degenerative changes in hepatocytes. Histopathological study of Liver from single dose test drug administered groups exhibited mild to moderate fatty changes and sinusoidal dilation. Liver sections from double dose test drug administered groups exhibited moderate fatty changes and sinusoidal dilation. (Figure 1)

Pancreas

Histopathological changes from positive control group showed moderate to severe degenerative changes, exhibited few islets of small size with much reduced granulation, cellularity was less, vacuolization was observed. (Figure 2)

In sections from test drug with single dose administered group- medium sized islets with medium cellularity and much reduced vacuolization was observed. In sections from test drug with double dose administered group- mild sized islets with moderate cellularity and reduced vacuolization was observed. Thus, changes in Liver and pancreas were found to be moderately significant indicating significant cellular protection of against diabetes pathology**.**

Conclusion

Diabetic mellitus (DM) is an endocrine disorder in which glucose metabolism is impaired, having multiple pathological facts including lifestyle. Traditional medicine claims about use of *G. abyssinica* Cass seed oil, said to be healthy nutritive. Today whole world is looking for safe, nutritive, healthy source of oils and fats which can be used in Diabetes. Hence this study has been planned to evaluate antidiabetic activity of *Guizotia abyssinica* Cass seed oil in Streptozotocin induced Wistar albino rats.

In this study drug showed the prominent decrease in the Serum Sugar level. Being the test drug itself the source of fatty acids, it has shown decrease in total cholesterol, LDL simultaneously not showed the significant increase in HDL. Histopathological observation of Liver and pancreas shown the safety aspect of the drug against cytotoxic effect of STZ. Thus, test drug proved as promising, protective, nutritive source of edible oil.

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