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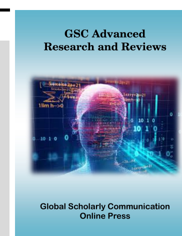

(RESEARCH ARTICLE)

In vitro and *in vivo* anti-diabetic activities of ethanolic leaf extracts of *Guizotia abyssinica*

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Abstract

Management of type 2 diabetes with an agent having no side effects is still a challenge for the researchers, however if the side effects are lessened & there may be a chance for reduced adverse reactions or severe side effects due to drug interaction. These interactions may be due to either any concomitant drug therapy or any dietary supplements taken together with the drugs. This study sought to evaluate the antidiabetic potentials of ethanolic extract of leaf of *Guizotia abyssinica (G. abyssinica)* in diabetic rats. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. The *in vitro* α-amylase inhibitory activity of the *G. abyssinica* was done by spectrophotometric method. The *in-vivo* method was performed by administering orally the ethanolic extract of *G. abyssinica* in streptozotocin-induced male Albino Wistar rats. Phytochemical analysis revealed the presence of alkaloids, glycosides, phenols, flavonoids, tannins. The study was compared using standard Glibenclamide (2 mg/kg body weight). The ethanolic extracts of *G. abyssinica* leaves exhibited the dose dependent α-amylase inhibitory activity with an IC_{50} value of acarbose and extract was found to 61 µg/ml and 75 µg/ml respectively. Oral treatment of ethanolic extract of *G. abyssinica* using rat oral needle at 200 and 400 mg/kg doses significantly ($P < 0.001$) decreased blood glucose levels in diabetic rats than control rats indicating that *G. abyssinica* is a promising source as a herbal medicine.

Keywords: Diabetes; *Guizotia abyssinica*; α-Amylase inhibitory activity; Streptozotocin; Glibenclamide**.**

1. Introduction

Diabetes mellitus (DM) is one of the most significant chronic metabolic disorders characterized by hyperglycemia. The vast majority of cases of diabetes fall into two broad etiopathogenetic categories. In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. In the other, much more prevalent category, type 2 diabetes causes is a combination of resistance to insulin action and an inadequate compensatory insulin-secretory response [1]. The overall prevalence of diabetes mellitus in the global population is approximately 6%, of which 90% is type 2 diabetes. India had 32 million diabetics in 2000, and this number is expected to increase to 80 million by 2030 [2, 3]. Characteristic of diabetes is associated with disturbances in the metabolism of carbohydrates, lipids and proteins due to defects in insulin secretion, insulin action or both [4]. Diabetic complications are nephropathy, retinopathy, neuropathy, atherosclerosis and fatty liver. In all these cases continual hyperglycemia plays a significant role in the induction of oxidative stress by increasing glucose autooxidation, nonenzymatic protein glycation and activation of polyol pathway [5]. Also hyperglycemia induced stress sensitive signaling pathways including nuclear factor (NF)-kB. Activation of NF-kB increased cytokine concentrations such as tumor necrosis factor-a (TNF-α). The renal cells are capable to synthesis TNF- α moreover the sensitive to changes of serum's TNF- α level. This process suggests a causal

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role for hyperglycemia in the immune activation of diabetes [6]. Since ancient times, consumption of medicinal herbs has considered in treatment of several diseases [7]. In recent years this kind of treatment has received growing attention because it is natural and has a few side effects [8]. *Guizotia abyssinica* (Asteraceae), is also known as noog/nug, Niger, nyger, nyjer or Niger seeds, ramtil or ramtilla; and blackseeds. It has good antifungal, antimicrobial, antitumor activities. Dutta et al., (1994) [9] studied the lipid composition of six cultivars of Ethiopian Niger and found that the total lipid was triacylglycerides and polar lipids accounted for 0.7- 0.8% of the total lipid content. Niger oil is also used in the manufacture of soap and paint and as a lubricant or lighting fuel. The remaining meal is used as feed, fertilizer, or fuel [10]. In North America, niger seed is used as a birdseed. Previous research on niger seed has focused on studying the nutritional makeup of the seed and methods of efficient propagation of the plant [11-13]. The whole seed contains up to 40% oil, 20.9% carbohydrate, and 27.8% protein. Therefore, the aim of this work was to determine the quality (types) of bioactive compounds and *In-vitro* and *In-vivo* anti-diabetic activity of ethanolic extract of *G. abyssinica*.

2. Material and methods

2.1. Pant material

The leaves of *G. abyssinica* were collected from Indore (M.P) and which was authenticated by Department of Botany, Govt. Holkar Science College, Indore (M.P).

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. Mumbai, India), SD Fine- Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

2.3. Extraction

Dried pulverized leaves of *G. abyssinica* were placed in thimble of soxhlet apparatus. Soxhlation was performed at 40-60ºC using petroleum ether as non-polar solvent at first. Exhausted plant material (marc) was dried and then extracted with ethanol. Soxhlation was continued till no colour was observed in siphon tube. For confirmation of exhausted plant marc (i.e. completion of extraction), colorless solvent was collected from siphon tube and completion of extraction was confirmed by absence of any residual solvent, The entire extract was concentrated to dryness using rotary flash evaporator under reduced pressure and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts [14].

2.4. Qualitative phytochemical analysis of plant extract

The *G. abyssinica* extracts obtained was subjected to the preliminary phytochemical analysis [15, 16]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

2.5. *In-vitro* anti-diabetic activity

2.5.1. Inhibition of alpha amylase enzyme

A total of 500 µl of test samples and standard drug (10-50 µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.

2.6. *In -vivo* anti-diabetic activity

2.6.1. Streptozotocin-induced anti-diabetic activity

Animal

Wistar rats (150-200 grams) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2°C, 55-65 %). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried

in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The studies were carried out with the approval of the Institutional Animal Ethics Committee (IAEC: SBRL/IAEC/PN-18041). The animals were fasted overnight and deprived of food for 16 hours, but they were allowed free access to water.

Acute oral toxicity

Acute toxicity study of the prepared extracts was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423 [17] the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals; and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, The dose level to be used as the starting dose is selected from one of three fixed levels 5, 300 and 2000 mg/kg body weight. Acute toxicity was determined as per reported method [18].

Induction of diabetes to animals

A single dose (100 mg/kg b.w., I.P.) of streptozotocin monohydrate dissolved in sodium citrate buffer was used for the induction of diabetes in rats after overnight fasting. After 1 hr of streptozotocin monohydrate administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

2.7. Experimental design

Five groups of rats six in each groups received the following treatment schedule for 14 days.

- GROUP I - Normal control (normal saline 10 ml /kg, P.O)
- GROUP II - Streptozotocin treated control (100 mg/kg, I.P)
- GROUP III - Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).
- GROUP IV - Streptozotocin (100 mg/kg, I.P) + EEGA (200 mg/kg, P.O)
- GROUP V - Streptozotocin (100 mg/kg, I.P) + EEGA (400 mg/kg, P.O)

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of rats at weekly intervals till the end of the study 1, 7, and 14 days.

2.8. Statistical analyses

Statistical analysis was done by using GRAPHPAD PRISM 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one-way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.

3. Results and discussion

Phytochemical analysis of ethanolic extracts of leaf sample of *G. abyssinica* showed the presence of flavonoid, phenols, alkaloid, protein, saponins and diterpines while, amino acid diterpines and carbohydrate were not detected Table 1. Percentage inhibition of α-amylase activity by *G. abyssinica* leaves extract was estimated with acarbose as the positive control, extract show dose dependent activity. IC_{50} value of acarbose and extract was found to 61 and 75 μg/ml respectively Table 2. As shown in Table 3 Blood glucose level of animals in all groups was recorded at 1, 7th and 21th day. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment Glibenclamide 2 mg/kgp.o., *G. abyssinica* 200 and 400 mg/kg/p.o. (112±2.8; 245.2±3.250and 162.1±1.8) treated group blood glucose level was decrease significantly ($p<0.05$) at 21st days, respectively.

Table 1 Phytochemical screening of extract of *G. abyssinica*

S. No.	Constituents	Ethanolic extract
1.	Alkaloids Mayer's Test Wagner's Test Dragendroff's test Hager's test	 +ve +ve +ve +ve
2.	Glycosides Modified Borntrager's Test Legal's test	 +ve +ve
3.	Flavonoids Lead acetate Alkaline test	 +ve +ve
4.	Phenolics Ferric Chloride Test	 +ve
5.	Proteins and Amino acids Xanthoproteic test Ninhydrin Test	 -ve -ve
6.	Carbohydrates Molisch's Test Benedict's Test Fehling's test	 -ve -ve -ve
7.	Saponins Froth Test Foam test	 +ve +ve
8.	Diterpins Copper acetate test	 -ve
9.	**Terpenoids** Lieberman Burchardt test	 +ve

Table 2 Results of *in vitro* antidiabetic studies of *G. abyssinica* extract

S. No	Acarbose		Ethanolic extract	
	Conc.	**% Inhibition**	**Conc.**	**% Inhibition**
1.	25	25	25	30
2.	50	45	50	36
3.	75	53	75	50
4.	100	56	100	56
5.	125	65	125	61
IC50 (µg/ml)		61	IC50	75

Table 3 Antidiabetic activity of *G. abyssinica* on blood glucose level in STZ-induced diabetic rats.

Groups	Treatment	Dose	Blood glucose (mg/dl)		
			Days 0	**Days 7**	**Days 21**
I	Normal	Normal saline	79.83±2.833	75.7±4.014	76.7± 4.944
II	Negative Control	Normal saline	265.2±3.85	270.1±2.9	275.2±2.5
III	Glibenclamide	2 mg/kg p.o.	255.83±2.386	135.63±3.8***	112±2.8***
IV	EEGA	200 mg/kg p.o.	260±3.5	250.3±3.138**	245.2±3.250**
V	EEGA	400 mg/kg p.o.	263±4.55	173.1±2.88***	162.1±1.8***

The values were expressed as Mean ± S.E.M. (n=6 animals in each group

4. Conclusion

The present study deals with the pharmacological and preliminary phytochemical analysis of an ethanolic extract of leaves of *G. abyssinica*. According to the phytochemical evaluation, it shows the presence of phyto-constituents like alkaloids, phenolic contents and flavonoids. Studies have reported that alkaloids and flavonoids play a key role in alleviating diabetes mellitus. Both *in-vitro* and *in-vivo* method of anti-diabetic activities were evaluated. The ethanolic extract of *G. abyssinica* showed a significant *in-vivo* anti-diabetic activity which was supported by the *in-vitro* study. This may be due to the presence of alkaloids and flavonoids present in the ethanolic extract.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest, financial or otherwise.

References

[1] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2005; 28: 37-42.

[2] Ramchandran A, Snehalatha C, Vijay V. Burden of type 2 diabetes and its complications – the Indian scenario. Current Science. 2002; 83: 1471-1476.

[3] Cowie CC, Eberhardt MS, editors. Diabetes: Vital Statistics. Alexandria: American Diabetes Association. 1996.

[4] Sivakumar S, Palsamy P, Subramanian SP. Impact of D-pinitol on the attenuation of proinflammatory cytokines, hyperglycemiamediated oxidative stress and protection of kidney tissue ultrastructure in streptozotocin-induced diabetic rats. Chemico-Biological Interactions. 2010; 188(1): 237-45.

[5] Saravanan G, Ponmurugan P. S-allylcysteine improves streptozotocin- induced alterations of blood glucose, liver cytochrome P450 2E1, plasma antioxidant system, and adipocytes hormones in diabetic rats. International Journal of Endocrinology. 2013; 11(4): e10927.

[6] Ingaramo PI, Ronco MT, Frances DE, Monti JA, Pisani GB, Ceballos MP, et al. Tumor necrosis factor alpha pathways develops liver apoptosis in type 1 diabetes mellitus. Molecular Immunology. 2011; 48(12-13): 1397–407.

[7] Nasiri A, Ziamajidi N, Behrouj H, Abbasalipourkabir R, Dehghan A. The effects of aqueous extract of chicory root on steatosis, lipid profile and liver damage enzyme markers in tamoxifentreated rats. Biochemistry & Molecular Biology Journal. 2014; 1(3): 185-94.

[8] Behrouj H, Ziamajidi N, Abbasalipourkabir R, Nasiri A, Solemani Asl S. Therapeutic effect of silybum marianum plant extracton tamoxifen-induced fatty liver in rats. Avicenna Journal of Medical Biochemistry. 2015; 3(1): e27160.

[9] Dutta PC, Helmersson S, Kebedu E, Alemaw G, Appelqvis LÅ. Variation in lipid composition of Niger seed (Guizotia abyssinica Cass.) samples collected from different regions in Ethiopia. Journal of the American Oil Chemists' Society. 1994; 71(8): 839-843.

[10] Vaughan JG. The Structure and Utilization of Oilseeds, Chapman & Hall, London. 1970; 41-43.

[11] Dagne K, Jonsson A. Oil content and fatty acid composition of seeds of guizotia cass (Compositae), Journal of the Science of Food and Agriculture. 1997; 73: 274–278.

[12] Gautam LP, Gautam SP. Biochemical studies on deoiled niger-seed cake of the ootakmond variety. Journal of Phytological Research. 1994; 7: 69-72.

[13] Sarvesh A, Reedy TP, Kishor BPK. Embryogenesis and organogenesis in cultured anthers of an oil yielding crop niger (guizotia abyssinica, cass). Plant Cell Tissue and Organ Culture. 1993; 35: 75-80.

[14] Nayak A, Garg M, Jain S, Khan MA, Jain DK, Khan N. Anti-Urolithiatic and *in vitro-in vivo* anti-oxidant effects of methanolic extract of thunbergia laurifolia on ethylene glycol-induced kidney calculi in rats. Scholars Academic Journal of Pharmacy. 2019; 8 (3): 94-104.

[15] Jain DK, Nayak A, Patel P, Jain A, Khan MA. Appraisal of *in vitro* antioxidant and in vivo anti-inflammatory activities of various extracts from the fruits of vitis vinifera L. Scholars Academic Journal of Pharmacy. 2019; 8 (3): 86-93.

[16] Jain DK, Patel NS, Nagar H, Patel A, Chandel HS. Anti-arthritic activity of tridax procumbens ethanolic extract of leaves. RGUHS Journal of Pharmaceutical Sciences. 2012; 2(4): 80-86.

[17] Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications. 1996.

[18] Jonsson M, Jestoi M, Nathanail AV, Kokkonen UM, Anttila M, Koivisto P, et al. Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. Food and Chemical Toxicology. 2013; 53: 27-32.