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Evaluation of hypoglycaemic activity of spine gourd powder (*Momordica dioica* Roxb.) formulated product (Khakra) in STZ induced diabetic rats

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Abstract

Spine gourd is popular as an emerging underutilized crop due to its various health benefits and medicinal properties. Its powder is used to form a dish called *Khakra*. *Khakra* is one of the famous dishes made out of mat bean and wheat flour, a popular dish of western India mainly in Gujarat and Rajasthan, which is used as a snack or during breakfast. During the preparation of *Khakra* along with whole wheat flour and Bengal gram flour, 50 % of spine gourd powder was used and evaluated for hypoglycaemic activity in STZ induced diabetic wistar rats. The oral administration of *Khakra* of about 5 to 10 g/rat/day was given for diabetic induced and normal rats for about 21 days and observations were recorded at 0th, 7th, 14th and 21st day for serum glucose (mg/dL), serum cholesterol (mg/dL) and serum haemoglobin (mg/dL). The Group 6 (STZ + Laboratory diet + Spine gourd *Khakra* at 10g/ kg body weight/day) showed significant decrease (P<0.01) in serum glucose, serum cholesterol and significant increase (P<0.01) in serum haemoglobin which was found to be dose-dependent. In case of normal rats no significant decrease in serum glucose and serum cholesterol was observed.

Key words: Spine gourd, *Khakra*, STZ, Wistar rats, hypoglycaemic activity

Introduction

Chronic metabolic disease in which the pancreas loses the ability to produce insulin or use the insulin produced. It is also a condition where blood glucose (blood sugar) levels rise. According to the WHO, diabetes affects 422 million people globally, making it one of the leading causes of death in low- and middle-income countries. Many hypoglycemic agents are available today, including insulin, but people prefer Ayurvedic or herbal medicines due to their low cost and lack of side effects. In many cases, people still use traditional medicinal plants to treat diseases.

The spine gourd (*Momordica dioica* Roxb.) is an emerging underutilized vegetable with immense nutritional and medicinal properties mainly used by tribal people. It is a perennial, dioecious climbing creeper belongs to the family Cucurbitaceae. It is widely distributed in Assam, the Garo Hills of Meghalaya, West Bengal, Uttar Pradesh, Bihar, Maharashtra, Madhya Pradesh, Gujarat and also southern parts of India and Andaman Islands. All parts of the plant are used for medicinal purpose, fruits have diuretic, hepatoprotective, alexiteric stomachic laxative, antivenum property and also it is used to cure asthma, leprosy, excessive salivation, prevent the inflammation caused by lizard, snake bite, elephantiasis, fever, mental disorders and digestive disorders. Fruits are cooked in a small amount of oil and consumed to treat diabetes (Bawara *et al*., 2010). *M. dioica* has potential therapeutic properties (antioxidant, vitamin, secondary metabolites), which help fight against diabetes, cancer, neurodegenerative diseases, and other life-threatening disorders (Talukdar and Hossain, 2014). *M. dioica* fruit extract possesses antidiabetic activity by increasing serum insulin, HDL and decreasing glycosylated haemoglobin and fasting blood glucose, postprandial glucose, total cholesterol, very low-density lipoprotein and low-density lipoprotein (Singh *et al.*, 2011). Phenolic compounds and flavonoids are responsible for reducing diabetes (Jain *et al.,* 2014).

In the present days, value addition is becoming more important because of its convenience, which makes seasonal produce available throughout the year, increases the storage life, and it reduces the time required to prepare dishes. According to so many case studies and literature available, it comes to the picture that spine gourd has anti-diabetic properties and many other medicinal properties. Apart from consuming raw vegetables, the effort is made to prepare spine gourd *Khakra*, a popular Gujarati snack food, and evaluate the hypoglycaemic property of spine gourd *Khakra* on STZ induced diabetic Wistar rats.

Materials and methods

Collection of plant material: The matured spine gourd fruits were collected from Sirsi, Karnataka, India in replicates**.** The plant was collected from agricultural land of Bagalkot, Karnataka. It was identified and authenticated [Ref No. B.Sc/Bot/102] by Mr. S A Kappali, Department of Botany, Basaveshwara Science College, Bagalkot-587101, Karnataka. The fruits were sorted, cleaned, and graded to remove damaged or diseased ones and to maintain uniform maturity, after which they were thoroughly washed with tap water and cut into 0.5 cm slices with a sharp knife. The slices, along with the seed, were air dried. The dried

spine gourd slices and seeds were weighed in an electronic balance and ground into powder using a food grinder. Spine gourd powder was sealed in high density polyethylene bags and used for further research.

Preparation of spine gourd *Khakra*: Whole wheat flour (30 mg), bengal gram flour (20 mg) and spinegourd powder (50 mg) were mixed well in a bowl. Other ingredients like cumin seeds (2 g), chilli powder (2 g), turmeric powder (1 g), milk (40 mL), asafoetida (50 mg), oil (5 mL) and salt (5 g) were added. Water and milk were added as required to the above mix and kneaded in to smooth and soft dough. The dough was covered with a plate and kept for 15-20 minutes. Oil was applied on its surface, divided it into equal parts and make it in to ball shape. Then ball was rolled out with pin into very thin circle on a wooden board. Dry wheat flour was dusted as much as required to prevent dough from sticking to board. *Khakra* was roasted both the sides on tawa under low heat for 20-30 seconds. Each part of *Khakra* was pressed down with cloth to make sure that no bubbles appear. Flipped it and the same process was repeated for the remaining dough. *Khakras* were cooled for 10-15 minutes at room temperature packed in HDPE bag, sealed and stored at ambient condition.

Induction of diabetes: Thirty six weanling male Wistar rats weighing 150-250 g (supplied by the animal house of the Department of Pharmacology, BVVS, Hanagal Shri Kumareshwar of College of Pharmacy, Bagalkot, Karnataka were used after obtaining approval from Animal Ethical Committee (Ref. No: IAEC/HSKCOP/April 2019/UHS 2). The rats were divided into six groups consisting of six rats maintained at room temperature with 12 h light/dark cycle. They were subjected for 5 days acclimatization period. All the groups were fed with water *ad libitum* for 3 weeks.

Experimental design: The rats were divided in to six groups and each group consisted of six rats.The first three groups *i.e.*, G_1, G_2 and G_3 were not induced with diabetes while the G_1 was given only the laboratory diet, the other two groups G_2 and G_3 were fed with two dose of spine gourd *Khakra* at 5 g and 10 g per kg body weight respectively along with laboratory diet. The other three groups G_4, G_5 and G_6 were induced with diabetes.

Streptozotocin (STZ; Sigma-Aldrich, USA) was dissolved in 0.1M sodium citrate buffer just prior to use and injected intraperitoneally to rats. Control rats (group I, n=9) received an equivalent volume of citrate Streptozotocin (STZ; Sigma-Aldrich, USA) dissolved in 0.1M sodium citrate buffer just prior to use and injected intraperitoneally to rats. Control rats (group I, n=9) received an equivalent volume of citrate Streptozotocin (STZ; Sigma-Aldrich, USA) dissolved in 0.1M sodium citrate buffer just prior to use and injected intraperitoneally to rats. Control rats (group I, n=9) received an equivalent volume of citrate.

Group	Treatment	Dose (g/rat/day)	No. of rats	Duration (days)
G_1	Laboratory diet (LD)	-	6	21
G_2	LD + Spine gourd *Khakra*	5	6	21
G_3	LD + Spine gourd *Khakra*	10	6	21
G_4	LD + STZ (control)	-	6	21
G_5	STZ + LD + Spine gourd *Khakra*	5	6	21
G_6	STZ + LD + Spine gourd *Khakra*	10	6	21

The group G_4 was considered as negative control where no test dosage was given. G_5 and G_6were fed with two dose of spine gourd *Khakra* at 5g and 10g per kg body weight respectively along with laboratory diet. Diabetes was induced by a single injection of 140 mg/ kg body weight of alloxanstreptozotocin (STZ) in 0.9 % w/v saline solution to overnight fasted rats. After 72 hrs. of the injection, the blood samples were drawn from retro-orbital sinus and the blood glucose levels were set on to conform the development of diabetes above 180 mg/dL. The dosage of spine gourd *Khakra* was given for 21 days daily once and blood glucose was determined for serum glucose level, serum cholesterol level and serum haemoglobin level on 0^{th}, 7^{th} and 21^{st} day.

Collection of blood samples: On 0^{th}, 7^{th} and 21^{st} day the blood was collected in clean, sterilized and labelled eppendorf tubes from experimental animals, by retro-orbital plexus puncture method using micro-haematocrit capillary tubes. Immediately after collection of blood into eppendorf tubes, the tubes were centrifuged at 3000 rpm for 10 min and held in a slanting position to facilitate serum separation at room temperature for 1-2 h. The clear non-haemolysed serum was then transferred into a clean, sterilized vial. The separated serum was used for analysis of serum glucose and serum cholesterol level.

Serum glucose profile (mg %): Estimation of serum glucose level in rats was done by Trinders method (Lott and Turner, 1975). Sufficient number of test tubes were labelled for each standard, control and sample to be tested. 500 µL of glucose reagent was pipetted into each test tube and 5 µL of sample was added to each tube. After each addition it was mixed well and incubated at 37º C for 10 min. Using cuvettes, absorbance was measured for each reaction mixture at 525 nm against water as a blank.

Serum cholesterol (mg %): PEG-CHOD-PAP method (Saikia *et al.,* 2016) was used for estimation of serum cholesterol. Sufficient test tubes were labelled for each standard, control and sample to be tested. 500 µL reagent was pippeted out and 10 µL of sample was added to each tube, mixed well and incubated at 37ºC for 10 min. or at room temperature (15-30ºC) for 30 min.The absorbance was read at 505 nm in UV spectrophotometer.

Serum haemoglobin (mg %): The haemoglobin (Hb) concentration in all blood samples was measured according to the cyanomethemoglobin method using Drabkin's reagent (Alexander and Grifiths, 1993). Blood samples were analysed for serum haemoglobin in City Diagnostic Centre, Bagalkot.

Statistical analysis: The data recorded in the experiment were subjected to completely randomised block design (CRD). One-way analysis of variance (ANOVA) followed by multiple comparison Tukey's test and $P < 0.05$ was considered statistically significant.

Results

Results of the effect of graded doses of spine gourd *Khakra* on blood glucose level of both normal and diabetic rats and comparison of significant results of diabetic induced treated groups (G5 & G6), including diabetic control (G4) against non-diabetic control group (G1) is given in Table 1. The spine gourd *Khakra* produced peak hypoglycemia. Dose dependent blood glucose reduction was observed in animals treated with 5, 10 g/ kg produced (23.13, 40.6 %, respectively). Diabetic control (G4)

showed a significant increased in glucose level as compared to non-diabetic control group (G1) and diabetic induced treated groups (G5 & G6) shows significantly decreased the glucose level as compared to non-diabetic control group (G1).

Table 1. Effect of spine gourd incorporated *Khakra* on serum glucose profile of STZ induced diabetic rats

Treatments	Serum glucose profile (mg/dL)		
	0th day	7th day	21st day
G_1	92.58±1.24	95.93±2.81	94.78±1.82
G_2	119.33±1.45	114.98±1.87	123.03±1.66[a]
G_3	90.55±1.72	85.40±1.57	104.09±1.45
G_4	286.05±1.62	280.30±1.51	289.38±1.82
G_5	199.68±1.92	200.88±1.42	153.48±2.79
G_6	198.48±1.88	176.10±1.72	118.45±3.18
SEM±	25	24.40	23.02
CD (at 1%)	101.78	99.34	93.71

G_1: Laboratory diet; G_2: Laboratory diet + Spine gourd powder *Khakra* (5g/rat/day); G_3: Laboratory diet + Spine gourd powder *Khakra* (10 g/rat/day); G_4: Laboratory diet+ Streptozotocin (control); G_5: Streptozotocin (STZ) + Laboratory diet + Spine gourd powder *Khakra*(5g/rat/day); G_6: STZ + Laboratory diet + Spine gourd powder *Khakra*(10 g/rat/day)

Results of the effect of the different doses of spine gourd *Khakra* on the serum cholesterol levels of both normal and diabetic rats and comparison of significant results of diabetic induced treated groups (G5 & G6), including diabetic control (G4) against non-diabetic control group (G1) is given in Table 2. The spine gourd *Khakra* at different dosage showed significant maximum reduction of serum cholesterol was observed in animals treated with 5, 10 g/ kg produced (13.70 %, 39.63 % respectively). This tabular column represents the diabetic control (G4) shows a significant increased in Serum cholesterol level as compared tonon-diabetic control group (G1) and diabetic induced treated groups (G5 & G6) shows significant decreased in Serum cholesterol level as compared to non-diabetic control group (G1).

Results of the effect of the different doses of spine gourd *Khakra* on the serum haemoglobin level are given in Table 3. The spine gourd *Khakra* at dosage of 5g/ kg shows significantly increased the serum haemoglobin level from 10.55 % to 14.55 % in the case of G6. This tabular column represents the diabetic control (G4) shows a significant decreased in Serum haemoglobin level as compared to non-diabetic control group (G1) and diabetic induced treated groups (G5 & G6) shows significant increased

Table 2. Effect of spine gourd incorporated *Khakra* on serum cholesterol profile of STZ induced diabetic rats

Treatments	Serum cholesterol profile (mg/dL)		
	0th day	7th day	21st day
G_1	29.58±2.76	30.65±1.87	33.00±1.76
G_2	28.78±2.61	26.00±1.76	24.93±1.78
G_3	24.80±1.78	23.63±1.68	22.20±2.87
G_4	60.38±2.76	54.78±1.13	44.15±1.56
G_5	53.10±2.81	49.25±2.78	38.10±1.56
G_6	51.85±2.81	46.00±2.01	26.65±2.87
SEm±	2.62	2.77	3.24
CD (at 1%)	10.68	11.28	13.17

All the values are expressed as mean ± SEM, n = 9, [a]$P<0.001$ as compared to control group and *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (One wayAnalysis of Variance [ANOVA] followed by multiple comparison Tukey's test) as compared to control group.

Table 3. Effect of spine gourd incorporated *Khakra* on serum haemoglobin profile of STZ induced diabetic rats

Treatments	Serum haemoglobin profile (%)		
	0th day	7th day	21st day
G_1	14.55±1.76	13.85±2.05	13.70±2.81
G_2	13.30±2.76	12.53±1.98	13.48±1.72
G_3	13.33±2.18	12.73±1.72	13.85±1.65
G_4	10.38±1.66	11.08±1.57	11.45±2.36
G_5	10.88±1.72	12.70±1.44	13.98±1.62
G_6	10.55±2.41	13.13±1.65	14.45±1.24
SEm±	0.45	0.33	0.31
CD (at 1%)	1.83	1.34	1.28

in Serum haemoglobin level on 7th and 21st day as compared to non-diabetic control group (G1).

Discussion

In the case of non-diabetic treated rats, the influence of *Khakra* showed variation in serum glucose profile. But not continuously reduced the glucose level, which says *Khakra* do not reduce the serum glucose profile below normal in non-diabetic person. Serum glucose profile in G4 (diabetic control) increased continually from 0 day to 21st day due to induction of diabetes by STZ, which causes hypoinsulinemia and hyperglycemia by damaging pancreatic β cells. In treatment G6 group, a higher percentage of serum glucose reduction can be seen followed by G5 group against G4 since a higher amount of spine gourd incorporated *Khakra* was given. This lowering effect of spine gourd incorporated *Khakra* may be due to activation of β-cells and granulation returns to normal giving insulinogenic (Kedar and Chakrabarti, 1982) effect. Aqueous extract of *M. dioica* effectively decreased the blood glucose in alloxan-induced diabetic rats up to 76.90 percent, which is better than the effects produced by standard drug glibenclamide (Singh *et al.,* 2011). *M. dioica* methanolic extract (MDMtE) in streptozotocin-treated diabetic rats markedly reduced serum glucose and increased serum insulin levels (Gupta *et al.,* 2011).

During diabetes, the excess glucose present in the blood reacts with haemoglobin to form glycosylated haemoglobin. So the haemoglobin level is decreased in diabetic rats [Al-yassin and Ibrahim (1981) and Sheela and Augusti (1992)]. Insulin is a potent inhibitor of lipolysis because it inhibits the activity of hormone-sensitive lipases in adipose tissue and suppresses the release of free fatty acids, which reduces the cholesterol level. This may be due to the effect of active flavonoids, phenols, steroids and saponins; these compounds may scavenge free radicals liberated by alloxan in diabetic rats (Rauter *et al.,* 2009).

In the present investigation, the oral administration of spine gourd *Khakra* to STZ induced male Wistar rats showed decreased reduction and normalization of elevated serum glucose and serum cholesterol with normalization of haemoglobin level compared to normal untreated rats. Therefore, it can be concluded that *Khakra* made out of spine gourd has shown hypoglycaemic activity in STZ induced Wistar rats.

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