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NOVEL ANTI-DIABETIC POTENTIAL OF "SUGAR CARE" A MIRACLE DRINK EVALUATED ON HUMAN DIABETIC SUBJECTS

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ABSTRACT: The herbal-based formulation "Sugar Care" a product of Miracle Drinks was assessed for an anti-diabetic property on 12 diabetic patients. Each patient was prescribed 15ml of Sugar Care (S10) morning and evening after meal and 15ml of cardiovascular care (S3) in the afternoon. Morning and night doses of sugar care were given along with 15ml of Liver Care (S4) and Renal Care (S5). After 30 days of treatment, the biochemical markers related to blood sugar, like fasting blood sugar (FBS), post-prandial blood sugar (PPBS), and haemoglobin-linked sugar or HBA1C were assessed. A significant reduction of FBS i.e., 50 to 72.2%, was observed in 5/12 diabetic patients. Female patients showed a better recovery rate than male patients, who were 3/4, and 3/8 patients, who showed more than 50% reduction in FBS. A similar recovery trend was also noticed in both male and female diabetic patients that showed a substantial decline in PPBS after treatment with Sugar Care. Three female patients showed substantial change from 346.0±6.0, 420.0±3.0 and 407.3±1.53mg/dl to 158.0±2.0, 194.0±2.0 and 189.0±3.0mg/ dl indicating 54.3, 53.8 and 53.6% decline in PPBS respectively. Among 12 patients treated, six patients showed more than a 50% decrease in blood PPBS. Sugar care-treated patients showed a 13.9-21.8% reduction in haemoglobin attached to sugar. Sugar care formulation showed no toxicity towards the Myoblast cell line. Glucose uptake assay evaluated on L6 cells at 250 and 500µg/ml of sugar care indicated 107.4 and 159.25% glucose uptake, respectively. Sugar Care showed significant anti-diabetic activity in human subjects and *in-vitro* cell lines.

INTRODUCTION: Glucose is a key nutrient for the living organism as it plays a diverse role in human metabolism. It is the main precursor for routine energy production in the cell, biosynthesis of the majority of macromolecules to build cell material and production of ATP currency of the body [1].

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Glucose homeostasis in humans is regulated mainly by insulin (produced from β-cells of the pancreatic islets of Langerhans) and glucagon, which produce higher Hyperglycemia and Hypoglycemia, respectively [2]. The impaired synthesis of insulin results in a chronic metabolic disorder called Diabetes mellitus.

Insufficient insulin secretion is caused by insulin resistance, low-level insulin, to a lesser extent, defective insulin receptors and signalling [3, 4]. There are two forms of diabetes Type 1 Diabetes Mellitus (T1DM) or insulin-dependent diabetes and Type 2 diabetes mellitus (T2DM) or insulin-independent diabetes [5]. While in the early stages, diabetes

mellitus patients are asymptomatic, in the long run, high blood glucose results in several complications such as neuropathy, nephropathy, retinopathy, cerebrovascular disease, and peripheral vascular disease reduce the quality of life [6, 7]. Today, diabetes mellitus affected most of the global population and is one of the serious global health concerns causing huge mortality and morbidity. Globally, Diabetes mellitus is a major cause of death among the top 10 diseases. The International Diabetes Federation (IDF) study on the global trend of Diabetes mellitus in 195 countries revealed that 451 million adults had diabetes in 2017 and was predicted to rise to 693 million by 2045 [8].

In India, 77 million diabetic patients were reported in 2019, and by 2045 it is anticipated to cross over 134 million cases, in which 57% remained undiagnosed [9]. T1DM is continuously rising, accounting for around 3 to 5% per year but not as that as T2DM. In 1 lakh children around 3 new cases of T1DM of age 0 to 14 years are reported and in contrast to Chennai and Haryana, Karnataka reported the highest T1DM cases [10, 11]. Anti-diabetic drugs available on the market include metformin, sulfonylureas, thiazolidinediones, acarbose, glimepiride, lispro, and as part are used for better management of diabetes mellitus by enhancing insulin sensitivity, stimulating glucose uptake, replacing insulin, and up-regulating insulin secretion. These drugs revealed several side effects; for instance, metformin is associated with lactic acidosis, diarrhea, and sulfonylurea has showed unwanted side effects like hypothyroidism, tachycardia, weight gain, and hepatic failure [12].

Further, diabetes-related complications and insulin resistance are constantly increasing [13]. Plants have been explored as huge sources of medicine for the treatment of various diseases, including Diabetes mellitus. As per World Health Organization (WHO), the primary health care need of about 80% of the population of the developing nations rely on plants derived products as their traditional medicine [14]. Among 21,000 plants enlisted by WHO for medicinal use purposes, 2500 plant species have Indian origin, and 800 plants are reported for anti-diabetic potential [15, 16]. Some of the plants reported for anti-diabetic activity include *Agrimonia eupatoria, Momordica charantia, Coccinia indica, Citrullus colocynthis, Azadirachta indica etc.* [16]. In the current investigation, the herbal formulation of Miracle Drink product "Sugar Care (S10)" was evaluated for anti-diabetic activity on 12 human subjects of ages ranging from 49 – 69 years. Dr. Raju S.M. meticulously prepared the formulation with immense experience in Ayurvedic and Neoayurvedic practice for around 35 years. Patients were advised to take Sugar Care for one month time. The effect of Sugar Care was evaluated by monitoring the patient blood for various biochemical parameters like Fasting Blood Sugar (FBS), Post prandial blood sugar (PPBS), and HBA1C before and after treatment. Sugar Care was further tested for cytotoxicity assay and anti-diabetic activity by glucose uptake assay on Rat Skeletal Myoblast cells (L6) cell lines.

MATERIALS AND METHODS:

Human Subjects: Confirmed diabetic patients of age between 49 to 69 years old consisting of both males and females were selected.

Miracle Product "Sugar Care": Sugar Care, a Miracle Drinks product, is mainly used as therapy for patients with abnormal glucose levels in the blood occurred due to insulin resistance. Sugar Care formulation consist mainly Guduchi, Jambu, Haritaki, Nimba, Arjuna, Bilwa, Brahmi, Amalakki, Gokshura, Ashwagandha, Chirayata, Karanja. Guduchi restores the function of β cells that synthesize insulin and control blood glucose and its anti-inflammatory and anti-arthritic potential reducing swelling, pain, and support in macro and microcirculation. Jumbo's anti-diuretic and hypoglycemic properties reduced the urination and sugar levels, respectively. The antioxidant potential of Jumbo also declines hyperglycemia. Haritaki is the drug of choice for diabetes and possesses antioxidant activity that helps boost the patients' immunity and promotes insulin sensitivity.

Cell Line Culture: Dulbecco's Modified Eagle Medium-High Glucose (DMEM-HG) medium added with streptomycin, penicillin, and amphotericin B at 100 µg/ml, 100IU/ml, and 5µg/ml, respectively were supplied with 10% inactivated Fetal Bovine Serum (FBS) was cultivated with Rat Skeletal Myoblast cells (L6) cell lines. The cell lines were incubated in 25 cm^2 culture flasks at 37°C with 5% CO_2 in a humidified atmosphere until confluence is formed. The

monolayer was trypsinized using TPVG (Trypsin Phosphate Versene Glucose Solution) solution containing 0.2% trypsin, 0.02% EDTA, and 0.05% glucose in phosphate buffer solution. Experiments were conducted in 96-well microtitre plates.

Sample Preparation for Cell viability Assay: in 1ml of DMEM-HG medium containing 10% FBS, 10mg of Sugar Care was added to get the stock of 10mg/ml. The stock solution was filter sterilized, and further serial two-fold dilution was prepared to obtain lower dilutions such as 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8 for cytotoxicity assay.

Anti-diabetic Treatment Protocol using Sugar Care: Diabetic patients confirmed by a detailed diagnosis procedure were selected for the study. Twelve human subjects with known diabetes were included in the study, ranging from 49 to 69 years old. Before initiation of therapy patients' FBS, PPBS and HBA1C was tested, and subjects were to undergo the treatment.

Each patient was prescribed 15ml of Sugar Care or S10 formulation morning and evening after meal and 15ml of cardiovascular care (S3) was given only in the afternoon. Morning and night doses of sugar care were given along with 15ml of Liver Care (S4) and 15ml of Renal Care (S5). The treatment was prescribed for one month, improvements in the biochemical parameters related to the blood sugar were diagnosed, and the anti-diabetic potential of Sugar Care was assessed.

Estimation of Biochemical Parameters: Followed by the treatment with Sugar Care for one month, and blood samples were collected from treated patients. The serum/plasma part was separated, and RBS and FBS content were estimated using the automated machine's help of the Span Diagnostic kits method.

However, PPBS from the blood was determined using the procedure mentioned in the Kit manual. Haemoglobin content of blood withdrawn from diabetic patients after one month treated of Sugar Care was estimated using Star 21 Semi-auto biochemistry analyzer supplied by Rapid Diagnostic Pvt. Ltd. Delhi.

MTT Dye-based Cell Viability Assay: the cytotoxicity of the Sugar Care formulation was tested on H9C2 cell lines from the concentration ranging from 7.8 to 1000µg/ml. Trypsinization of a monolayer of cell culture was carried out, and cell counts were adjusted to $1x10^5$ cells/ml of MDEM-HG containing 10% FBS. In 96 well microtitre plates, 0.1ml of diluted cell suspension was taken in each well and incubated for 24hours. After incubation, a partial monolayer formed was washed, and the supernatant was removed. The partial monolayer was treated with 100µl of different concentrations of the test drug, and incubation of the plate was carried out at 37°C with a 5% CO_2 atmosphere for 24h. Following the incubation, the medium containing the drug was discarded, and 50µl of phosphate buffer saline containing MTT dye was added to each well. Incubation was continued for another 3 hours in the same condition. After incubation, the supernatant was discarded, 100µl DMSO was added to dissolve formazan, and absorbance was recorded at 540nm. The percentage of cell growth by 50% (CTC_{50}) was calculated from the dose-response curve.

Study of Anti-diabetic Activity by *In-vitro* Glucose Uptake Method: The non-toxic concentration of the test drug was used for glucose uptake assay on L6 cells showing 70 – 80% confluence maintained in Petri plate containing DMEM with 2% FBS. Cells were starved with serum overnight, and before the experiment, cells were once washed with HEPES solution buffered with KRP (Krebs Ringer Phosphate solution), and cells were incubated at 37°C with 0.1% BSA for 30 minutes.

The different non-toxic concentration of drug 250 and 500µg was used for the treatment of cells, and 10mM Metformin and cell controls were maintained as a positive and negative control, respectively. Cells were incubated for 30 minutes at 37°C by adding 20µl of D-glucose to each well simultaneously. Following the incubation, the assay was terminated through solution aspiration from each well, and using ice-cold KRP buffered solution, cells were washed trice. Cell lysis was carried out using 0.1M NaOH solution, and cell-associated glucose in cell lysate was determined using a glucose assay kit supplied by ERBA. The percentage of glucose-uptake enhancement compared to controls was determined by recording two independent experimental values.

RESULTS AND DISCUSSION: The present study assesses Miracle Drink's product, i.e., Sugar Care, for anti-diabetic activity by selecting 12 human diabetic patients. Well-known diabetic patients were treated with 15ml of Sugar Care drink morning and evening and 15ml of cardiovascular care scheduled only in the afternoon. In conjugation with the main treatment, 15ml of Renal Care and Liver care were prescribed morning and evening, along with Sugar Care. Therapy was continued for one month, and patients were tested for all biochemical parameters like RBS, FBS, PPBS, and haemoglobin content. The test results were discussed in the following paragraphs.

Effect of Sugar Care on FBS: Diabetic patients treated with Sugar Care for one-month treatment showed significant improvement in FBS compared to 0^{th}-day treatment. The details of FBS in diabetic patients after one treatment and the percentage of reduction in FBS were tabulated in **Table 1.** The data were very diverse, and five patients showed a substantial change in FBS concentration, as indicated in **Fig. 1.0.**

FBS determined on 0^{th} day of treatment of these patients drastically declined from 386.0±2.00, 316.7±3.06, 231.7±3.06, 245.7±2.52 and 296.7±1.53 to 107.3±1.53, 91.0±2.00, 99.0±1.00, 110.0±2.00, and 148.3±3.51 which corresponded to 72.2, 71.3, 57.3, 55.2 and 50.0 percentage decrease in FBS respectively.

The remaining diabetic patients showed less than a 50% reduction in FBS during one treatment with the Sugar Care formulation. Although no age and sex correlation with treatment was noticed, females showed better recovery from diabetes, as half of the patients treated with Sugar Care showed more than 50% reduction in their FBS.

Whereas, out of 8 male patients, three revealed more than 50% decline in Sugar Care in one month of treatment. The results concluded that Sugar Care of Miracle Drink's product showed significant anti-diabetic activity when tested on human subjects diagnosed with diabetes.

TABLE 1: FBS OF DIABETIC PATIENTS BEFORE AND AFTER TREATMENT WITH SUGAR CARE

Human Subject	Age (Years)	Sex	Concentration of FBS in Blood (mg/dl)		% Reduction
			0^{th} Day Treatment	30^{th} Day Treatment	
Patient 1	55	M	131.7±2.52	94.7±4.16	28.1
Patient 2	53	M	158.7±3.06	122.7±2.51	22.7
Patient 3	57	M	316.7±3.06	91.0±2.00	71.3
Patient 4	59	F	315.7±3.51	186.3±1.53	41.0
Patient 5	56	M	231.7±3.06	99.0±1.00	57.3
Patient 6	50	F	296.7±1.53	148.3±3.51	50.0
Patient 7	52	M	312.3±3.51	216.0±2.00	30.8
Patient 8	50	F	116.7±1.53	85.3±0.58	26.9
Patient 9	54	F	245.7±2.52	110.0±2.00	55.2
Patient 10	67	M	183.7±1.15	140.0±2.00	23.8
Patient 11	69	M	270.3±1.53	172.0±2.00	36.4
Patient 12	49	M	386.0±2.00	107.3±1.53	72.2

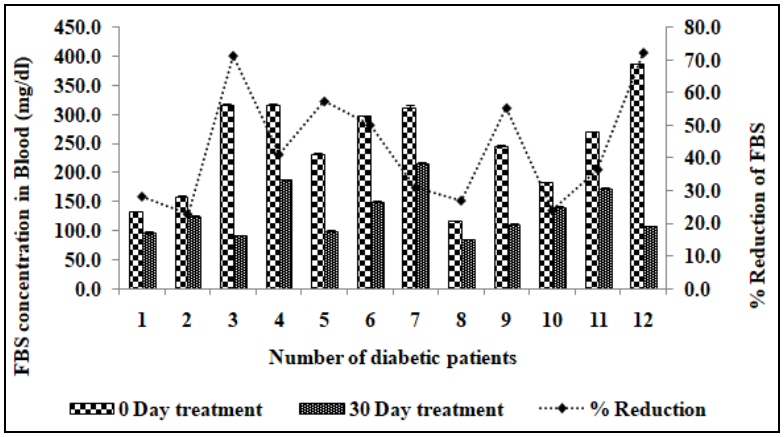

FIG. 1: STUDY OF THE EFFECT OF SUGAR CARE ON FBS OF DIABETIC PATIENTS

The Trend of PPBS during Sugar Care Treatment: PPBS is an important parameter to be considered during diabetic management. PPBS are blood glucose levels after about 2 hours of the meal, and it should be less than 140mg/dl in non-diabetic patients and less than 180mg/dl in diabetic patients. In the present investigation, diabetic patients with high PPBS tested before treatment were selected and prescribed one-month treatment with Sugar Care formulation. The results obtained were shown in **Table 2.** The PPBS level of diabetic patients was considerably reduced in both male and female patients. Out of four female patients, PPBS of three patients of age 54, 59 and 50 years showed a considerable change from 346.0±6.0, 420.0±3.0, and 407.3±1.53 mg/dl to 158.0±2.0, 194.0±2.0 and 189.0±3.0mg/dl that associated with 54.3, 53.8 and 53.6 % decline in PPBS respectively. On the contrary, the recovery rate in male diabetic patients during one treatment with Sugar Care was relatively low compared to female diabetic patients *i.e.*, 3/8 diabetic patients showed more than 50% reduction in blood PPBS. The 49-year-old patient indicated the highest reduction in PPBS, with a 56.4% reduction. Analysis of complete data of one month of Sugar Care treatment indicated a substantial recovery rate where 50% of diabetic patients *i.e.*, 6/12 revealed a decline in blood PPBS as shown in **Fig. 2**. Hence, Sugar care showed significant anti-diabetic potential when tested on human diabetic patients with no noticeable side effects.

TABLE 2: EFFECT OF SUGAR CARE ON PPBS OF THE DIABETIC PATIENT DURING ONE MONTH OF THERAPY

Human Subject	Age (Years)	Sex	Concentration of FBS in Blood (mg/dl)		% Reduction
			0^{th} Day Treatment	30^{th} Day Treatment	
Patient 1	55	M	323.7±2.08	254.0±4.00	21.5
Patient 2	53	M	198.3±3.51	156.0±2.00	21.3
Patient 3	57	M	500.7±4.04	220.0±2.00	56.1
Patient 4	59	F	420.0±3.00	194.0±2.00	53.8
Patient 5	56	M	274.0±2.00	128.0±2.00	53.3
Patient 6	50	F	407.3±1.53	189.0±3.00	53.6
Patient 7	52	M	514.3±1.53	287.0±3.00	44.2
Patient 8	50	F	184.0±2.00	120.0±4.00	34.8
Patient 9	54	F	346.0±6.00	158.0±2.00	54.3
Patient 10	67	M	205.3±6.51	160.7±3.06	21.8
Patient 11	69	M	416.0±4.00	234.0±2.00	43.8
Patient 12	49	M	453.7±2.08	197.7±4.04	56.4

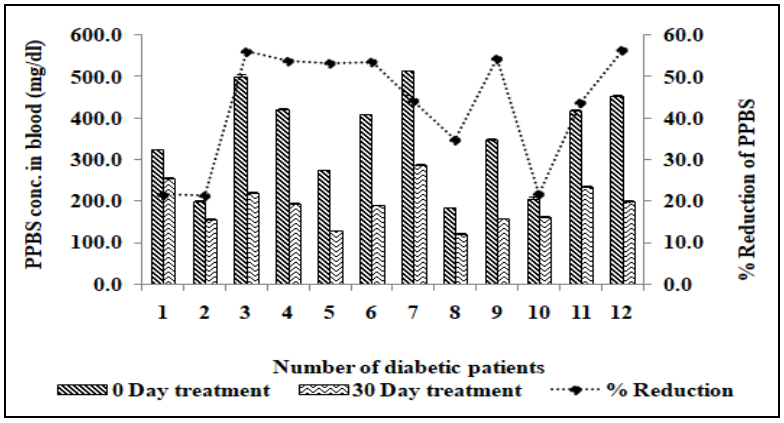

FIG. 2: ESTIMATION OF PPBS IN SUGAR CARE TREATED DIABETIC PATIENTS

Haemoglobin attached Sugar or HBA1C: Diabetic patients treated with Sugar Care were also evaluated for total blood sugar attached to the haemoglobin. The long-term blood glucose was measured based on the HBA1C biomarker, and the normal range of HBA1C in healthy individuals was found to be below 6%. Hence, HBA1C was tested as a maker to know the sugar concentration of all selected diabetic patients before and after Sugar Care therapy, and results were recorded in **Table 3**. Diabetic patients treated with Sugar care for one month time showed a 13.9 to 21.8% reduction in haemoglobin attached sugar. The amount of haemoglobin detected on 0^{th} day of treatment, *i.e.*,

8.6±0.29% to 7.0±0.12% was decreased to 7.40±0.11% to 5.50±0.10% as indicated in **Fig. 3**. The experimental observations indicated that Sugar Care can play an important role in managing blood sugar levels in diabetic patients. Additionally, since it is herbal-based, Sugar care could provide natural-based therapy with no side effects.

TABLE 3: LEVEL OF HBA1C A HAEMOGLOBIN-ATTACHED SUGAR IN DIABETIC PATIENTS TREATED WITH SUGAR CARE

Human Subject	Age (Years)	Sex	Concentration of HBA1C (%)		% Reduction
			0^{th} Day Treatment	30^{th} Day Treatment	
Patient 1	55	M	7.4±0.10	5.90±0.10	20.3
Patient 2	53	M	7.0±0.12	5.50±0.10	21.8
Patient 11	57	M	8.6±0.29	7.40±0.11	13.9

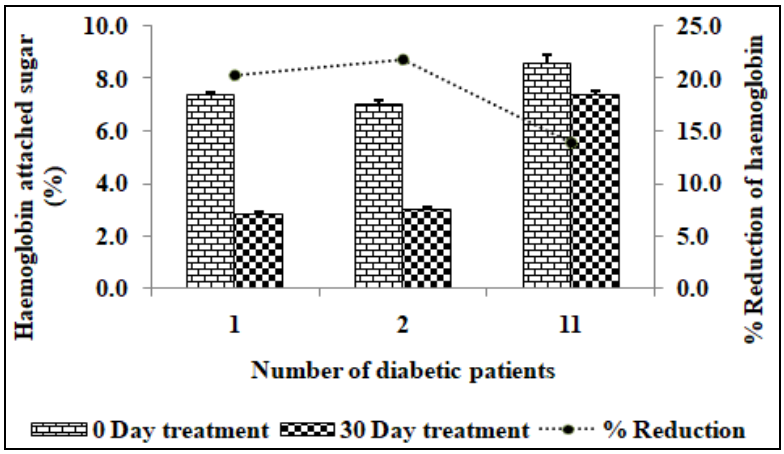

FIG. 3: ESTIMATION OF HBA1C AFTER TREATMENT WITH SUGAR CARE IN DIABETIC PATIENTS

Anti-proliferative Assay of Sugar Care (Safety Evaluation Study): Sugar care was evaluated for cytotoxicity on the Myoblast cell line (H9C2) from 7.8 to 1000 µg/ml, and results are recorded in **Fig. 4** and **Table 4**.

TABLE 4: STUDY OF CYTOTOXICITY OF SUGAR CARE TESTED ON AGAINST H9C2 CELL LINE

Test Conc. (Mg/Ml)	% Cytotoxicity	Ctc50 (Mg/Ml)
1000	20.38±2.24	
500	19.52±2.11	>1000
250	17.63±1.55	
125	11.41±2.75	
62.5	11.65±2.91	
31.25	8.18±2.64	
15.625	9.76±2.76	
7.8	7.04±2.15	

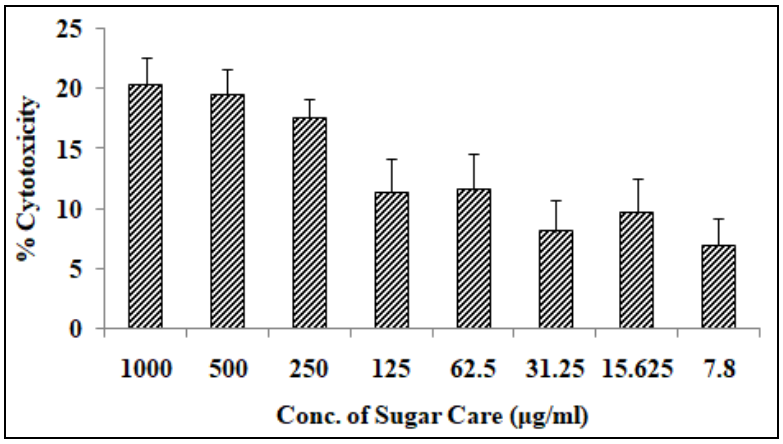

FIG. 4: ANTI-PROLIFERATIVE ACTIVITY OF SUGAR CARE TESTED AGAINST H9C2 CELL LINE

The cytotoxicity of Sugar Care was observed against the H9C2 cell line was increasing with an increase in the concentration of formulation. Anti-proliferation potential of Sugar care on H9C2 cells was seen at a minimum level, *i.e.,* more than 20.38% cell viability was observed in the highest dilution (1000 µg/ml) of Sugar care. So, the Sugar Care formulation was found to be safe for H9C2 cells at the tested concentration, and the cytotoxicity concentration (CTC_{50}) required to inhibit 50% of the cell population was >1000µg/ml. Hence, based on the cytotoxicity results, 250 and 500µg/ml of Sugar Care was selected for glucose uptake assay on L6 cell lines.

Glucose Uptake Assay on L6 Cell Lines: *In-vitro* based anti-diabetic assay was performed in L6 cell lines using a nontoxic concentration of Sugar Care at 250 and 500µg/ml, and results are recorded in **Table 5**. Metformin at 10mM was maintained as positive control and cell control as negative control. Sugar Care formulation at 250 and 500µg/ml indicated significant anti-diabetic activity by glucose uptake in L6 cell lines.

The activity was dose-dependent, and % of glucose uptake by L6 cells was found to be 107.4 and 159.25% at 250 and 500µg/ml of Sugar Care in contrast to 277% glucose uptake by positive metformin used at 10mM. The negative control revealed 100% glucose uptake. Glucose uptake assay results indicated significant anti-diabetic activity of Sugar Care at tested concentrations.

TABLE 5: *IN-VITRO* GLUCOSE UPTAKE STUDIES FOR TEST SUBSTANCE IN L6 CELL LINE

Sample Name	Sample Conc. (µg/ml)	% Glucose uptake over control
Sugar Care	500	159.25
	250	107.4
Metformin	10mmol	277
Cell control	-	100

Anti-diabetic study of Sugar care in an in-vitro model and assessed on human subjects resulted in substantial deceased in the blood sugar parameters. Patients treated with sugar care showed significant improvement in their diabetic symptoms. The experimental outcome of the Sugar care test indicated promising drugs for future anti-diabetic applications and disease management.

DISCUSSION: Diabetes mellitus has emerged as a major global health concern reported in most nations. The disease imposed a huge economic burden on the human healthcare system as it is associated with huge mortality, morbidity, and costly hospital management [17]. Currently, diabetes incidences are rapidly increasing all over the world. The disease poses a great threat to human beings for several reasons like the number of diabetic cases constantly increasing and the emergence of insulin resistance. In addition to adult diabetes, pediatric diabetes also rising [18, 19]. Hence, there is an immediate need for novel therapy for diabetes to prevent further damage caused by the disease. Currently, there is no complete cure for diabetes; however, disease can be better managed by adopting different lifestyle measures like diet and exercise. Low carbohydrate diet intake by diabetic patients helps in controlling hyperglycaemia and other side effects like constipation and headache. Regular exercise improves that glycaemic control by declining the body weight of diabetic patients and the well-being of the patients [20]. Presently, treatment methods used for diabetes treatment are composed of obesity treatment, lifestyle change, use of metformin (insulin sensitizer) and biguanide (reduce insulin resistance) which are used as first-line therapy. Additionally, other medications like insulin, inhibitors of alpha-glucosidase, thiazolidinediones and non-sulfonylurea secretagogues are also used [21].

Plant-based medicines are the best choice for a long time as they provide a complete cure and display no side effects. The sugar Care formulation tested in the present research revealed promising anti-diabetic activity during a one-month treatment duration. Biochemical parameters indicated the blood sugar levels of diabetic patients were significantly changed after the treatment with Sugar care. The formulation showed potential anti-diabetic activity, where half of the patients enrolled in the study showed a 50% reduction in blood sugar levels. Anti-diabetic activity studied in the *in-vitro* study showed excellent glucose uptake compared to

the standard control samples. Hence, based on the experimental evidence, one can conclude that Sugar care can be used as a substitute for insulin therapy. Further, the present formulation plays a very important role in the management of diabetes as well as in reducing hospital expenses for diabetic patients as these herbal-based formulations are very economical where all laymen can accesses the therapy.

CONCLUSION: Sugar Care, an herbal formulation of Miracle Drinks prepared from Guduchi, Jambu, Haritaki, and other important plants, was investigated for its anti-diabetic potential in 12 diabetic patients. Patients were advised to one month of treatment with Sugar care. An analysis of biochemical parameters thereafter indicated a significant reduction in their blood sugar levels, such as FBS, PPBS, and hemoglobin-attached sugar (HBA1C). About half of the diabetic patients included in the study revealed more than 50% of reduction in blood sugar, and female patients showed excellent recovery compared to the male diabetic patients. *In-vitro* anti-diabetic assay of sugar care showed noticeable glucose uptake over control by L6 cells. The results further supported the anti-diabetic potential of Sugar care formulation tested on human subjects. The sugar care formulation showed excellent anti-diabetic activity, which can be a promising candidate for the ideal drug development process in the future. As formulation ingredients improve the function of β cells, sugar care can be explored as an alternative therapy for patients under insulin treatment. Sugar care can play a significant role in managing diabetes and preventing its related complications.

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CONFLICTS OF INTEREST: Nil

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