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Development of Low-calorie Yoghurt with *Stevia rebaudiana* Powder and Study of Biological Feeding Trial using Wistar Rats

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Abstract

The investigation was conducted to determine the feasibility of incorporating stevia powder by replacing cane sugar at 25, 50, 75, and 100% levels for the manufacture of yoghurt. Concerning the rat feeding trial, rats fed with low-calorie yoghurt can able to maintain the body weight as that of the control group. The blood glucose level of group 2 was increased significantly than the normal control. The other control groups and also produced a marked decrease in blood glucose levels in diabetic rats after 28 days of treatment. The plasma insulin levels are significantly decreased from normal control and other treatment groups. Haemoglobin levels of G2 are considerably lower in the treatment group than in the normal control group. G2's HBA1c levels for the normal control and other treatment groups are significantly higher. The total protein levels of G2 in the treatment group are significantly lower than in the control group and other treatments. The total cholesterol levels of G2 in the treatment group are significantly greater than in the control group. A significant ($p<0.05$) reduction in triglycerides and a significant increase in HDL as compared to the diabetic groups was found. The hepatic functions analysis of low-calorie yoghurt-fed rats showed a significant reduction in the ALT, AST, and ALP levels and an increase in urea and creatine levels in diabetic control animals. The SOD, CAT, GPx, GSH and MDA levels of experimental rats after administration of low-calorie yoghurt samples showed a significant fall in the measured antioxidants in parenchymal cells of the liver. The histological examination of the pancreas revealed the presence of peripheral widening between the islets of Langerhans and pancreatic acini in the diabetic control group. However, diabetic rats that underwent treatment with various formulations of low-calorie yoghurt (100 mg/kg) showed relatively less pronounced architectural changes and low peripheral broadening between acinar and Langerhans cells as compared with diabetic rats. The summary of the research showed that oral administration of fermented milk products tasted with low-calorie stevia produced a reasonable improvement and protection in type 2 diabetic-induced lab animals without many side effects.

***Key words*:** Low-calorie yoghurt, *Stevia rebaudiana* powder, Wistar rat feeding trial

Globally, yoghurt is considered a healthy food due to its high digestibility and bioavailability of nutrients and also can be recommended for people with lactose intolerance, gastrointestinal disorders such as inflammatory bowel disease and irritable bowel disease, and aids in immune function and weight control. Besides the nutrient profile and has a rich source of bio-available calcium [1]. In the present lifestyle, the use of cane sugar poses health-related issues including diabetes and obesity. Due to growing health awareness, there has been a huge demand for sugar substitutes that would provide lesser or no calories and possess better sweetening potency. Similarly, the yoghurt prepared with cane sugar can be replaced with herbal sugar substitutes using *Stevia rebaudiana* powder. Stevioside and rebaudioside are the sweetest glycosides present in stevia leaves, which are 250–300 times sweeter than sucrose, and are chemically and thermally stable [2]. There is a stimulation of insulin secretion from β cells of islets of Langerhans and INS-1 cells by the direct action of stevioside and steviol [3]. Considering the beneficial characteristics, stevia leaf extract has been authorized as commercial sweeteners and food additives [4] as the safety of high-purity steviol glycosides has been extensively reviewed in the published literature and by national and international food safety agencies [5]. To improve the health benefits of yoghurt, the study is aimed to replacement of cane sugar by incorporating stevia powder at different levels and to study the physicochemical, microbiological properties and sensory evaluation of the yoghurt samples.

MATERIALS AND METHODS

The fresh raw Milk was purchased from the Arul Anandar College Dairy Farm, Karumathur and the spray-dried skim milk was purchased from Aavin, Madurai. A Commercial Lyophilized *Yoghurt Starter Culture (Streptococcus thermophilus and* Lactobacillus delbrueckii Subsp. *bulgaricus* (NDRI-YH-S And YH L) were purchased from NDRI, Karnal.

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The stevia powder was purchased from MAKa Foods, Madurai. Good quality cane sugar purchased from the local supermarket, Madurai. The stabilizer gelatine was purchased from Saraswathy Essence Mart, Madurai.

For the preparation of yoghurt, cow milk, skim milk powder (2%, w/v), sugar (6w/v), and gelatine 1%, w/v) were mixed in a boiler as per the treatment schedule in Table 1 to increase the milk solids to 17 per cent and this mixture sugar was added at the rate of 6 per cent to have a total solids content of 23 per cent. This mixture was heated at 85°C for 30 min in a double jacketed vat and left to cool to 42 °C. Milk was inoculated with 2 per cent of yoghurt *cultures Streptococcus thermophilus* and *Lactobacillus bulgaricus* at the ratio of 1:1 at 42 ± 1°C. The milk was incubated at 42 ± 1 °C until it reached pH 4.7 [6]. After the coagulum was cooled to room temperature and kept for 18 – 24 h at refrigeration temperature (4°C) before evaluation. To optimize the formula the trials were conducted with six replications.

Table 1 Ingredients for the preparation of low-calorie yogurt

Ingredients	TC	T1	T2	T3	T4
Milk (ml)	855	855	855	855	855
Skim milk powder (g)	85	98.5	112	126.5	145
Sugar (g)	60	45	30	15	0
Stevia (g)*	0	1.5	3.0	4.5	6.0
Gelatin(mg)**	0.5	0.5	0.5	0.5	0.5
Total	1000	1000	1000	1000	1000

*Stevia has 10 times more sweetener than common sugar, so its reduced quantity meets the taste of the product [31]

*Gelatin is not considered for the figuring of the ice cream mix.

Biological feeding trial using Wistar rats

Wistar rat feeding trial for glycemic index

Male Wistar rats weighing (190-200g) were obtained from the Central Animal House, K.M. College of Pharmacy. The experimental protocol was approved by the Institutional Ethical Committee of K.M. College of Pharmacy (IAEC/S.P.MALARKANNAN/MKU/21RDS516/KMCP/179/ 2022-23). They were housed in ventilated cages and fed with a normal pellet diet and water ad libitum. After overnight fasting, diabetes was induced by intraperitoneal injection of Streptozotocin (STZ) (Sigma Hypogly) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg. The control rats received the vehicle alone. The animals were allowed to drink a 5 per cent glucose solution overnight to overcome the drug-induced hypoglycaemia. After 1 week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycaemia (blood glucose range of above 220 mg/dL) were considered diabetic rats and used for the experiment. The rats were divided into seven groups of 4 animals in each group as follows: Group I: Normal control administered with 0.9 per cent sodium chloride (NaCl), Group II: STZ-induced diabetic control administered with 0.9% NaCl, Group III: Diabetic rats administered with low energy yoghurt formulation (T1) (100 mg/kg) through orally for 28 days and Group IV: Diabetic rats administered with low energy yoghurt formulation (T2) (100 mg/kg) through orally for 28 days, Group V: Diabetic rats administered with low energy yoghurt formulation (T3)(100 mg/kg) through orally for 28 days, Group VI: Diabetic rats administered with low energy yoghurt formulation (T4) (100 mg/kg) through orally for 28 days, Group VI: Diabetic rats administered with glibenclamide (2.5 mg/kg) orally for 28 days. Glibenclamide was suspended in 0.9% NaCl in warm water as a vehicle solution and administered orally for 28 days.

Biochemical blood analysis

The fasting blood glucose was measured on 0, 14 and 28 days by commercial kit. Body weight and plasma insulin by Enzyme-Linked Immunosorbent Assay (ELISA) kit were measured on 0 and 28 days [30]. After 28 days of treatment, the rats were fasted for 16 h and then sacrificed by cervical decapitation. The blood samples were collected in the tubes containing potassium oxalate and sodium fluoride as anticoagulants for the estimations of total haemoglobin [10], HbA1C [11] and total protein [7].

The body weight gain of individual rats in each group was estimated on weekly basis during the experimental period with individual diet using electronic weighing balance (KERN 440-35 N). Blood was collected retro-orbitally from the inner canthus of the eye under light ether anaesthesia using capillary tubes (Micro Hematocrit Capillaries, Mucaps). Blood was collected in fresh vials containing sodium fluoride and sodium oxalate as anti-coagulant:anti-glycolytic agents and plasma were separated in a T8 electric centrifuge (Remi Udyog, New Delhi) at 2000 revolutions per minute for 2 minutes.

Fasting blood glucose level was estimated by O-toluidine method [8]. A solution of orthotoluidine in glacial acetic acid when treated with glucose produces a blue-coloured product with an absorption maximum of about 640nm. The values obtained were representing the glucose level.

The level of plasma insulin was measured by the method of [9]. Haemoglobin is estimated by cyanmethoglobin method [10] Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide, the latter converts Haemoglobin to methoglob which is converted to cyanomethemoglobin by potassium cyanide. The absorbance of a solution is then measured in a spectrophotometer of a wavelength of 540nm. HbA1c in the blood was estimated by [11] method. For estimating the hepatic functions (ALT, AST and ALP), renal functions (urea and creatinine), lipid profiles (total cholesterol, triglycerides and high-density lipoprotein), enzymatic (SOD, CAT and GPx) and non-enzymatic antioxidants (GSH) and lipid peroxidation (MDA) were analysed using FIA 8000 Quantitative immunoassay analyser by Getein biotech Inc.

Histopathological study

The pancreatic tissues of the tested rats were fixed in 10% V/V formaldehyde, dried out in an evaluated arrangement of ethanol and embedded in paraffin. Pancreatic sections (5 µm thick) were acquired utilizing rotary microtome and afterwards rehydrated. Sections were then stained by Haematoxylin– Eosin (H&E) and viewed under the light microscope to assess the presence of β cells in the pancreatic tissue of various group. Digital images were obtained using an OlympusBX51 microscope equipped with a Camedia C3040ZOOM digital camera (Olympus America Inc., Melville, NY, USA). All images were taken under 40x magnifications [12]. All the values of body weight, fasting serum glucose, and biochemical estimations were expressed as mean ± SEM and ANOVA was carried out followed by Newmann Keuls multiple range tests using graph pad instat 3.0 statistical software. Differences between groups were considered significant at ($P<0.01$) levels.

RESULTS AND DISCUSSION

Biochemical analysis – Animal study for glycemic index using Wistar rats

Estimation of Bodyweight

No significant difference between the control and treatment groups except for the diabetic control group, showing a significant difference indicating that feeding rats with low-calorie yoghurt was able to maintain the body weight as that of the control group feed with a normal diet (Table 2). On the 28th day and 14th day G2 was significantly different from normal control G1. In the current study, diabetic rats fed with various formulations of low-calorie yoghurt (100 mg/kg) significantly gained weight, most likely due to reversing the glycogenolysis and gluconeogenesis and thereby helping the restoration of normal metabolic pathways [13].

Table 2 Estimation of body weight
(Effect of Various formulations of low-calorie yoghurt (100 mg/kg) on bodyweight in Streptozotocin and Nicotinamide induced Type-2 diabetes in rats)

Groups	Treatment	Body weight		
		0 Day	14 Day	28 Day
G1	Normal Control	215±5.5	230±6.4	244±6.3
G2	Diabetic Control	218±5.8	195±4.5*a	170±4.0*a
G3	Treatment Control	220±6.2	234±6.6	240±6.8
G4	Treatment Control	214±5.2	228±6.0	250±7.0
G5	Treatment Control	226±6.5	238±6.4	252±7.2
G6	Treatment Control	223±6.1	235±6.3	247±6.7
G7	STD control	222±6.0	233±6.2	252±6.9

Values are expressed as Mean±SEM
*a Values are significantly different from normal control

Estimation of blood glucose

A significant difference ($P<0.01$) between the control and treatment groups was found. The blood glucose level of group 2 was increased than the normal control and other control groups. Various formulations of low-calorie yoghurt (100 mg/kg) with the incorporation of stevia at various levels produced a marked decrease in blood glucose levels in diabetic rats after 28 days of treatment. The antidiabetic effect of various formulations of low-calorie yoghurt (100 mg/kg) may be due to the increased release of insulin from the existing beta-cells of the pancreas. Our findings are in agreement with those reported by [14] for the evaluation of the hypoglycemic and anti-hyperglycemic potential of Tridax procumbens.

Table 3 Estimation of blood glucose
(Effect of Various formulations of low-calorie yoghurt (100 mg/kg) on Blood Glucose in Streptozotocin and Nicotinamide induced Type-2 diabetes in rats)

Groups	Treatment	Blood glucose (Mg/Dl)		
		0 Day	14 Day	28 Day
G1	Normal Control	93.8±3.1	95.2±3.7	96.0±3.4
G2	Diabetic Control	254.5±6.8	278.4±7.5*a	292.5±7.8*a
G3	Treatment Control	247.4±6.2	185.4±5.4*b	172.8±4.9*b
G4	Treatment Control	250.5±5.8	175.4±5.0*b	150.2±4.3*b
G5	Treatment Control	245.2±5.4	178.7±4.7*b	155.3±4.0*b
G6	Treatment Control	240.3±5.1	172.5±4.3*b	148.4±3.9*b
G7	STD control	252.4±6.0	148.2±4.0*b	132.4±3.2*b

Values are expressed as Mean±SEM
*a Values are significantly different from normal control
*b Values are significantly different from Diabetic Control

Estimation of Plasma Insulin, HB, HBA1c and Total protein

A significant decrease in plasma insulin levels from normal control and other treatment groups has been found. There was found to be a substantial reduction in fasting plasma insulin levels in diabetic rats. These results are similar to the characteristic findings of nicotinamide / streptozotocin-induced type 2 diabetes mellitus in rats [15]. The current study's findings demonstrate a considerable enhancement of insulin concentration with a significant decline in fasting glucose levels in polyherbal formulation-treated diabetic rats.

The haemoglobin level of experimental rats after administration of low-calorie yoghurt revealed that haemoglobin levels of G2 are considerably lower in the treatment group than in the normal control group. During diabetes, the excess glucose present in the blood reacts with haemoglobin to form HbA1c [13]. Feeding of various formulations of low-calorie yoghurt (100 mg/kg) treated diabetic rats significantly increased total haemoglobin which might be the result of an improvement in glucose metabolism. The mean value of the HBA1c (Glaciated Haemoglobin) level of experimental rats after administration of low-calorie yoghurt indicated that G2's HBA1c levels from the normal control and other treatment groups are significantly higher. Feeding of lab animals with various formulations of low-calorie yoghurt (100 mg/kg) to diabetic rats showed a significant decrease in the level of HbA1c which might be the result of an improvement in the glucose metabolism [16].

Further, the total protein levels of G2 in the treatment group are significantly lower than in the control group. [17] reported that the serum total protein level in diabetic control rats was significantly reduced. An increase in serum protein, that is, the ratio of albumin and globulin in diabetic rats treated with an aqueous extract of *P. ligularis* and standard drug, was observed that decreased the level of liver markers in diabetic treated rats. [18] used the Sidr fruit pulp along with fermented camel milk-fed rats provoked a significant decrease in total protein compared to the control group.

Table 4 Estimation of Plasma Insulin (microlitre/ml), HB (mg/dl), HBA1c (%) and Total protein (g/dl)
(Effect of Various formulations of low-calorie yoghurt (100 mg/kg) on Plasma insulin in Streptozotocin and Nicotinamide induced Type-2 diabetes in rats)

Groups	Treatment	Plasma Insulin (microlitre/ml) 28thday	HB (mg/dl)	HBA1c (%) Glaciated Haemoglobin	Total protein (g/dl)
G1	Normal Control	19.45±0.80	13.7±1.8	6.18±0.78	4.82±0.43*a
G2	Diabetic Control	5.80±0.40*a	8.4±0.7*a	12.30±1.8*a	6.47±0.62*b
G3	Treatment Control	13.5±0.74*b	12.4±0.9*b	8.55±0.90*b	7.07±0.84*b
G4	Treatment Control	14.2±0.85*b	12.7±1.1*b	8.45±0.87*b	6.86±0.68*b
G5	Treatment Control	13.7±0.77*b	12.9±1.3*b	8.24±0.82*b	7.15±0.90*b
G6	Treatment Control	13.8±0.80*b	13.1±1.5*b	8.12±0.74*b	7.46±0.94*b
G7	STD control	17.15±0.95*b	13.4±1.7*b	7.22±0.65*b	8.08±0.70

Values are expressed as Mean±SEM
*a Values are significantly different from normal control
*b Values are significantly different from Diabetic Control

Lipid profile analysis - Total cholesterol, triglycerides, HDL, level

The cholesterol levels of G2 in the treatment group are significantly greater than in the control group. [17] reported that a significant elevation in serum lipids was observed in diabetic rats when compared with control rats. In the case of insulin deficiency as in diabetes mellitus, lipolysis is not inhibited and therefore this leads to hyperlipidemia. Oral administration of *Passiflora ligularis* fruit extract to diabetic rats for 30 days significantly reversed these values to near normal. This may be due to the increase in insulin secretion by *Passiflora ligularis* which decreases the total cholesterol.

Consecutive oral intake of various formulations of low-calorie yoghurt (100 mg/kg) for 28 days resulted in a significant ($P<0.05$) reduction in triglycerides of treatment controls as compared to diabetic control. [19] reported that there was a significant ($P<0.01$) increase in the level of serum triglycerides in diabetic rats as compared to the control group. The concentrations of lipids, such as triglycerides were significantly high in diabetic rats compared to the control group. On the evaluation of the antihyperglycemic, lipid-lowering and antioxidant effect of *Lactobacillus casei* and *Bifidobacterium bifidum* in streptozotocin (STZ)-induced diabetic rats.

Consecutive oral intake of various formulations of low-calorie yoghurt (100 mg/kg) for 28 days resulted in a significant increase in High-Density Lipoprotein (HDL) compared to the diabetic groups. Defective lipolysis is a common feature of T2DM that may be manifested by Low HDL-C [20]. In our study, HDL-C was significantly low in the NTD group and the present finding is consistent with [21] who induced T2DM through an STZ-high fat diet.

Table 5 Estimation of Total cholesterol, Triglycerides and HDL
(Effect of Various formulations oflow-calorie yoghurt (100 mg/kg) on Total cholesterol in Streptozotocin and Nicotinamide induced Type-2 diabetes in rat)

Groups	Treatment	Total Cholesterol(mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)
G1	Normal Control	145±5.45	83.8±2.85	47.5±2.35
G2	Diabetic Control	275±8.20*a	178.3±5.37*a	24.7±1.68*a
G3	Treatment Control	197±6.95*b	122.7±4.32*b	30.5±1.85*b
G4	Treatment Control	184±6.20*b	95.9±3.55*b	33.10±2.18*b
G5	Treatment Control	190±6.45*b	112.4±4.05*b	34.6±1.96*b
G6	Treatment Control	187±6.24*b	98.1±3.40*b	35.20±2.22*b
G7	STD control	168±6.05*b	89.4±3.08*b	37.50±2.45*b

Values are expressed as Mean± SEM
*a Values are significantly different from normal control
*b Values are significantly different from Diabetic control

Hepatic functional analysis - Estimation of Alanine transaminase, Aspartate transaminase (AST), Alkaline Phosphatase (ALP)

Successive oral intake of various formulations of low-calorie yoghurt (100 mg/kg) for 28 days resulted in a significant ($p<0.05$) reduction in ALT. The elevation in liver enzymes is characteristically associated with the glycemic status in type 2 diabetic patients [22]. In this study, various liver enzymes such as ALT were elevated in diabetic rats. The elevated transaminases might contribute to the evolution of diabetic ketogenesis and gluconeogenesis [23]. Alternatively, in diabetic rats various formulations of low-calorie yoghurt (100 mg/kg) treatment significantly reduced liver transaminase activity.

Oral intake of various formulations of low-calorie yoghurt for 28 days resulted in a significant ($P<0.05$) reduction in AST. In this study, various liver enzymes such as AST were elevated in diabetic rats. The elevation in liver enzymes is characteristically associated with the glycemic status in type 2 diabetic patients [16]. The elevated transaminases might contribute to the evolution of diabetic ketogenesis and gluconeogenesis [23]. Alternatively, in diabetic rats various formulations of low-calorie yoghurt (100 mg/kg) treatment significantly reduced liver transaminase activity.

Repeated oral intake of various formulations of low-calorie yoghurt (100 mg/kg) for 28 days resulted in a significant ($p<0.05$) reduction in ALP. The elevation in liver enzymes is characteristically associated with the glycemic status in type 2 diabetic patients [22]. In this study, various liver enzymes such as ALP were elevated in diabetic rats. The elevated transaminases might contribute to the evolution of diabetic ketogenesis and gluconeogenesis [23]. Alternatively, in diabetic rats, a different combination of stevia-incorporated low-calorie yoghurt (100 mg/kg) treatment significantly reduced liver transaminase activity.

Table 6 Estimation of Alanine transaminase, Aspartate transaminase (AST), Alkaline Phosphatase (ALP), (Effect of Various formulations of low-calorie yoghurt (100 mg/kg) on ALT in Streptozotocin and Nicotinamide induced Type-2 diabetes in rat)

Groups	Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)
G1	Normal Control	28.4±2.12	48.5±3.35	123.5±4.85
G2	Diabetic Control	57.3±3.75*a	95.6±5.20*a	240.2±7.33*a
G3	Treatment Control	42.5±3.05*b	59.4±4.62*b	185.4±6.25*b
G4	Treatment Control	39.3±2.85*b	57.8±4.55*b	165.7±4.95*b
G5	Treatment Control	41.5±2.95*b	58.1±4.28*b	180.7±6.08*b
G6	Treatment Control	42.2±3.00*b	56.3±4.17*b	168.6±5.15*b
G7	STD control	33.6±2.27*b	50.9±3.50*b	148.2±4.65*b

Values are expressed as Mean± SEM

*a Values are significantly different from normal control

*b Values are significantly different from Diabetic control

Renal functional analysis - Urea and Creatinine

The total protein levels of G2 in the treatment group are significantly greater than in the control group. The tested results are presented in Table 6 . In renal dysfunction that is induced by diabetic hyperglycemia, the serum urea level is markedly elevated (Yu and Bonventre, 2018) leading to impairment of the kidney in filtering the toxic or waste products out of the body. Furthermore, the serum urea level was considerably diminished in various formulations of low-calorie yoghurt (100mg/kg) treated diabetic rats, suggesting a renoprotective effect on diabetic rats. A significant increase in creatinine level was noticed (Table 7) which indicates a renal dysfunction that is induced by diabetic hyperglycemia, the serum creatinine level is markedly elevated [24]. The level of creatinine in the body suggests the level of impairment of the kidney in filtering toxic or waste products out of the body. Furthermore, the serum creatinine level was considerably diminished in the administration of various formulations of low-calorie yoghurt (100mg/kg) treated diabetic rats, suggesting a renoprotective effect on diabetic rats.

Table 7 Estimation of urea and creatinine
(Effect of Various formulations of low-calorie yoghurt (100 mg/kg) on Urea in Streptozotocin and Nicotinamide induced Type-2 diabetes in rat)

Groups	Treatment	Urea (MG/DL)	Creatinine (MG/DL)
G1	Normal Control	32.3±1.75	1.05±0.30
G2	Diabetic Control	83.75±3.50*a	3.05±0.75*a
G3	Treatment Control	57.8±2.58*b	1.45±0.48*b
G4	Treatment Control	51.4±2.24*b	1.32±0.42*b
G5	Treatment Control	55.4±2.45*b	1.48±0.55*b
G6	Treatment Control	52.3±2.28*b	1.37±0.47*b
G7	STD control	44.5±1.98*b	1.15±0.35*b

Values are expressed as Mean± SEM

*a Values are significantly different from normal control

*b Values are significantly different from Diabetic control

Enzymatic and Non-enzymatic antioxidant parameters - SOD (Unit/mg Protein), CAT (Mmol/min/mg Protein), GPx (Mmol/min/mg Protein), GSH (Mm/100mg tissue), MDA (Mmol/100mg tissue)

The SOD level (Unit/mg Protein**)** of experimental rats after administration of low-calorie yoghurt had a significant difference for normal control and groups (Table 8). Significant falls in the measured antioxidants were noted in the parenchymal cells of the liver [25]**.**

The CAT (mmol/min/mg protein) level of experimental rats after administration of low-calorie yoghurt had a significant difference for normal control and groups. The major falls in the measured antioxidants were noted in the parenchymal cells of the liver. The tested results were presented in Table 8. [26] observed a decline in the activity of SOD and CAT antioxidant enzymes together with a reduction in GSH levels described in diabetic animals

The Glutathione Peroxidase (GPx) (mmol/min/mg protein) level of experimental rats after administration of low-calorie yoghurt had a remarkable change. Significant falls in the measured antioxidants were noted in the parenchymal cells of the liver. [27] reported that groups of rats with hepatitis that were fed a yoghurt drink fortified with golden berry juice exhibited a significant increase in liver GPx levels compared with the hepatitis-positive control rats. [19] reported that the antioxidant indexes in the pancreas of diabetic rats returned to normal level elevation in glutathione peroxidase (55.56%, 72.23%, and 97.23%) on treatment with *L. casei*, *B. bifidum*, and combination.

The Glutathione (GSH) (mmol/100mg tissue) level of experimental rats after administration of low-calorie yoghurt for normal control and groups are presented in Table 8. Significant falls in the measured antioxidants were noted in the parenchymal cells of the liver. [27] reported that groups of rats with hepatitis that were fed a yoghurt drink fortified with golden berry juice exhibited a significant increase in liver GSH levels compared with the hepatitis-positive control rats. [19] reported that the antioxidant indexes in the pancreas of diabetic rats returned to normal level elevation in reduced glutathione (104.5%, 161.34%, and 179.04%) on treatment with *L. casei*, *B. bifidum*, and combination treatment. The current research findings are closely related to the report of [19] on the study of the antihyperglycemic, lipid-lowering and antioxidant effect of Lactobacillus casei and Bifidobacterium bifidum in streptozotocin (STZ)-induced diabetic rats.

The malondialdehyde (MDA) level (mmol/100 mg tissue) of experimental rats after administration of low-calorie yoghurt for normal control and groups are presented in Table 8. A significant ($P<0.05$) rise in MDA. [28] reported that there was a rise in the MDA level in diabetic Mellitus patients in this

study also in there was a raise of MDA notice but the level of raise comparatively less when compared with that of the diabetic control group indicating that the addition of stevia indicated that MDA was considerably diminished rats fed with low-calorie yoghurt incorporated with various percentage of stevia (100mg/kg) in treated diabetic rats.

Table 8 Estimation of SOD (Unit/mg Protein), CAT (Mmol/min/mg Protein), GPx (Mmol/min/mg Protein), GSH (Mm/100mg tissue), MDA (Mmol/100mg tissue

Groups	Treatment	SOD Unit/mg Protein	CAT Mmol/min/mg Protein	GPx Mmol/min/mg Protein	GSH Mm/100mg Tissue	MDA Mmol/100mg tissue
G1	Normal Control	9.22±0.88	93.8±3.48	10.8±0.90	58.25±3.25	1.20±0.28
G2	Diabetic Control	4.75±0.45*a	40.5±2.80*a	5.22±0.45*a	25.20±1.35*a	2.32±0.45*a
G3	Treatment Control	6.92±0.59*b	61.7±3.05*b	7.38±0.65*b	42.25±2.70*b	1.64±0.38*b
G4	Treatment Control	7.45±0.70*b	64.3±3.35*b	8.15±0.75*b	45.75±2.85*b	1.40±0.32*b
G5	Treatment Control	7.15±0.60*b	62.4±3.18*b	7.85±0.70*b	43.60±2.74*b	1.68±0.42*b
G6	Treatment Control	7.22±0.65*b	63.8±3.24*b	8.30±0.78*b	44.80±2.80*b	1.44±0.35*b
G7	STD control	8.70±0.85*b	84.5±3.85*b	9.20±0.85*b	53.90±3.10*b	1.34±0.20*b

Values are expressed as Mean± SEM

*a Values are significantly different from normal control

*b Values are significantly different from Diabetic control

Histopathological analysis of pancreas

The histological examination of the pancreas of the normal rats illustrated the normal architecture of islets of Langerhans, which were evenly distributed in the pancreatic tissue with different sizes in the same lobule of the pancreas. Each islet was arranged in an astomosing cellular plates and a reticular membrane was separated from each acinus. Alternatively, the pancreas of the diabetic control group revealed the presence of peripheral widening between the islets of Langerhans and pancreatic acini (Plate 1). In the glibenclamide-treated group, Langerhans cells were densely arranged, with insignificant space between adjacent cells and the absence of inflammatory cells. Architectural disarray was noted in the pancreas to some extent when compared to diabetic control animals. However, diabetic rats that underwent treatment with various formulations of low-calorie yoghurt (100 mg/kg) showed relatively less pronounced architectural changes and low peripheral broadening between acinar and Langerhans cells as compared with diabetic rats. Overall, various formulations of low-calorie yoghurt (100 mg/kg) showed maximum recovery from all histopathological changes with a partial proliferation of β-cells (Plate 1A - IG).

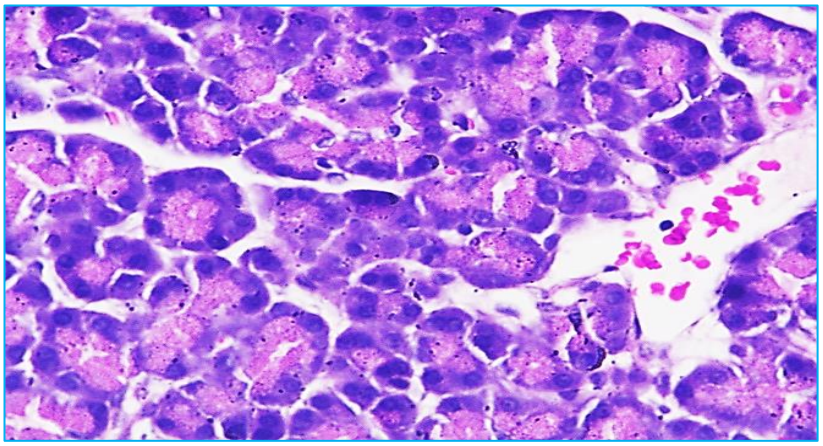

Plate 1 A: G1- Normal control (administered with 0.9% sodium chloride (NaCl) showing normal appearance of the pancreas)

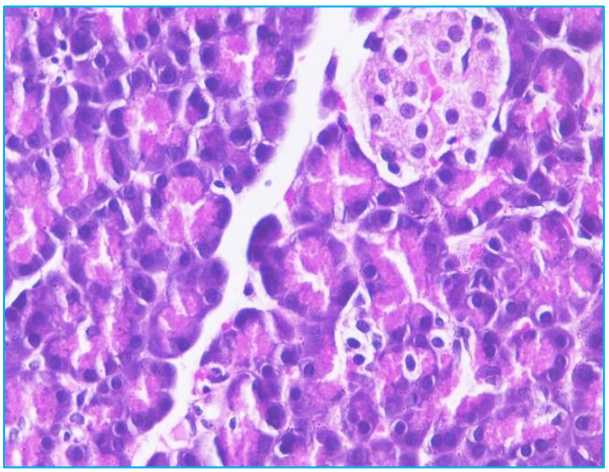

Plate 1B: G2-Diabetic control (administered with 0.9% NaCl group revealed pathological changes in parenchymal cells of pancreatic tissue)

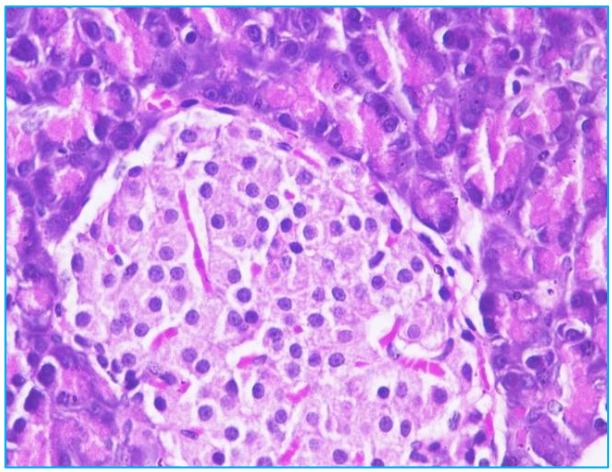

Plate IC: G3-Treatement Control (administered with low-calorie yoghurt formulation (T1) (100 mg/kg) orally for 28 days, showing a nearly normal architecture of the pancreas)

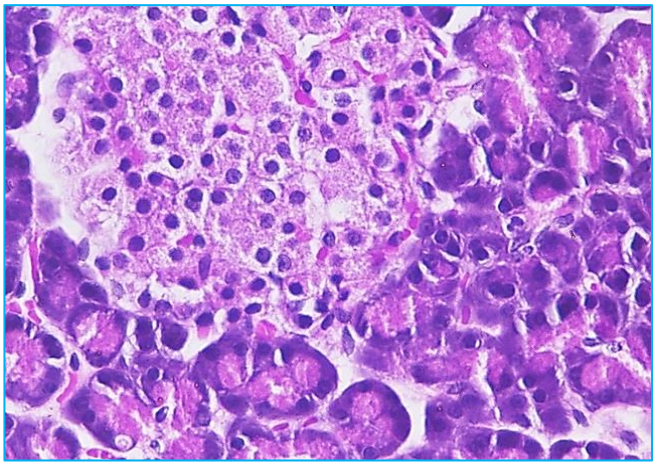

Plate ID: G4-Treatment Control (administered with low-calorie yoghurt formulation (T2) (100 mg/kg) orally for 28 days, showing a nearly normal architecture of the pancreas).

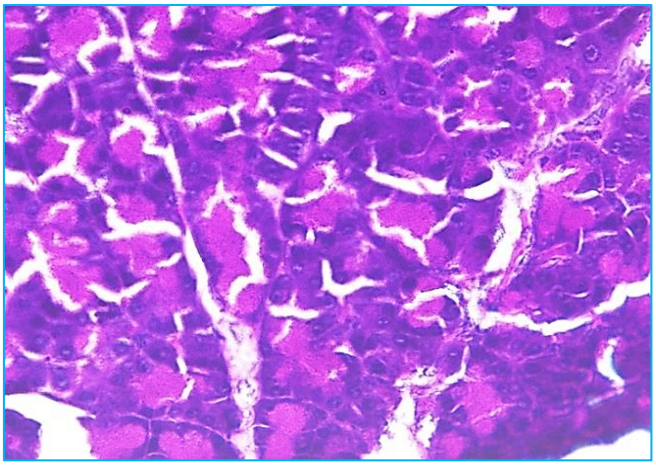

Plate IE: G5-Treatment Control (administered with low-calorie yoghurt formulation (T3) (100 mg/kg) orally for 28 days, showing a nearly normal architecture of the pancreas).

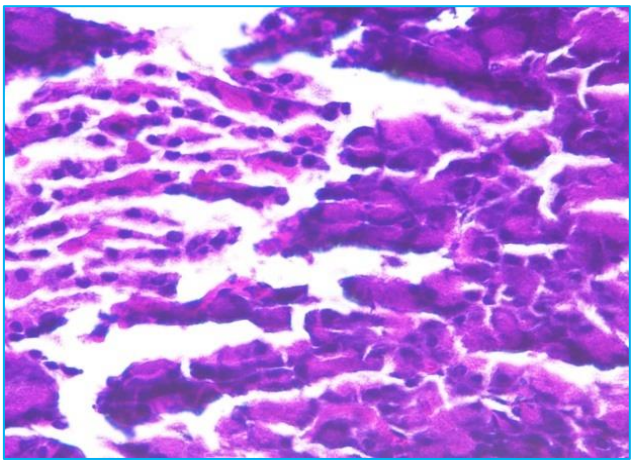

Plate IF: G6 – Treatment Control (administered with low-calorie yoghurt formulation (T4) (100 mg/kg) orally for 28 days, showing the nearly normal architecture of the pancreas).

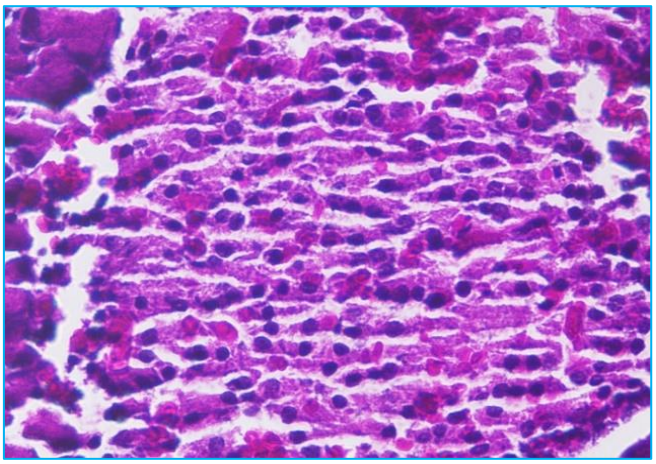

Plate IG: G7 – STD Control (administered glibenclamide (2.5 mg/kg) orally for 28 days, displaying restoration of the general architecture).

Plate I Histopathology of pancreas

The photomicrograph of the diabetic pancreatic tissue clearly shows the streptozotocin-nicotinamide-induced damage in both exocrine and endocrine components of pancreatic tissue. Glibenclamide stimulates pancreatic islet regeneration and is responsible for the increase in plasma insulin as observed during biochemical evaluations and the histological photomicrograph. The tested results were depicted in Plate A-G. [29] reported that the addition of probiotic culture and natural sweetener stevia through feeding low-calorie yoghurt appears to bring about repair/regeneration of the endocrine pancreas and hepatic cell protection in diabetic rats. It was observed that the various formulation of low-calorie yoghurt (100 mg/kg) showed protective activity against ROS-mediated damage, which occurs in the islets of Langerhans cells of the pancreas. The antidiabetic and antioxidant properties of stevia incorporated in various formulations of low-calorie yoghurt (100 mg/kg) may be attributed to the synergistic action of the phytoconstituents.

CONCLUSION

A detailed investigation was conducted to determine the feasibility of incorporating stevia powder by replacing cane sugar at 25, 50, 75, and 100% levels for the manufacture of yoghurt. The biological trial carried out with regard to the rat feeding trial, rats fed with low-calorie yoghurt can able to maintain the body weight as that of the control group with a normal diet. The blood glucose level of group 2 was increased significantly than normal control and other control groups and also produced a marked decrease in blood glucose levels in diabetic rats after 28 days of treatment. The plasma insulin levels are significantly decreased from normal control and other treatment groups. Haemoglobin levels of G2 are considerably lower in the treatment group than in the normal control group. G2's HBA1c levels for the normal control and other treatment groups are significantly higher. The total protein levels of G2 in the treatment group are significantly lower than in the control group and other treatments. The total cholesterol levels of G2 in the treatment group are significantly greater than in the control group. Consecutive oral intake of various formulations by rats for 28 days resulted in a significant ($p<0.05$) reduction in triglycerides and a significant increase in HDL as compared to the diabetic groups. The hepatic functions analysis of low-calorie yoghurt-fed rats the liver function tests showed a significant reduction in the ALT, AST, and ALP levels and an increase in urea and creatine levels in diabetic control animals. The SOD, CAT, GPx, GSH and MDA levels of experimental rats after administration of low-calorie yoghurt samples showed a significant fall in the measured antioxidants in parenchymal cells of the liver. The histological examination of the pancreas revealed the presence of peripheral widening between the islets of Langerhans and pancreatic acini in the diabetic control group. Architectural disarray was noted in the pancreas to some extent

when compared to diabetic control rats. However, diabetic rats that underwent treatment with various formulations of low-calorie yoghurt (100 mg/kg) showed relatively less pronounced architectural changes and low peripheral broadening between acinar and Langerhans cells as compared with diabetic rats. Overall, various formulations of low-calorie yoghurt (100 mg/kg) showed maximum recovery from all histopathological changes with a partial proliferation of β-cells. The summary of the research showed that oral administration of fermented milk products tasted with low-calorie stevia produced a reasonable improvement and protection in type 2 diabetic-induced lab animals without many side effects.

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