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Original Paper

Anti-diabetic Impact of Momordica charantia Seed and Aloe barbadensis Miller Gel Powder in Sprague Dawley Rats

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Abstract

Diabetes mellitus is the 4th leading cause of death worldwide. Diet-based approaches treating various health disorders are considered safe, economical, and sustainable. Complementary and alternative medicine (CAM) is one of the methods used worldwide for the management of diabetes millets. The present study was designed to achieve the following objectives: to analyze bitter melon seed (BMS) and aloe vera gel (AVG) powder for proximate and mineral content; to develop functional chicken nuggets by using various concentrations of both powdered and their compositional analyses and to probe the antidiabetic potential of powders in synergy. A control and two formulations were selected, based on consumer acceptability, for efficacy trial through the animal model of 30 days. For this purpose, 18 diabetic Sprague Dawley Rats were randomly divided into 3 groups. The groups included the positive control group (G0, normal diet) and two other groups with different AVG and BMS powder. G1 group received 50% BMS and 50% AVG powder whereas, G2 was fed on 75% BMS and 25% AVG powder per day. G1 showed a significant reduction in fasting blood glucose level, glycated haemoglobin, cholesterol, LDL-Cholesterol, blood urea nitrogen, and uric acid, whereas the G2 showed improvement in insulin, HDL-Cholesterol, and triglycerides levels whereas showed better control over time. Supplementation of BMS and AVG can help to control hyperglycemia and related complications.

Keywords: Aloe Vera, Gel, Momordica charantia, Complementary and Alternative Medicine (CAM).

Introduction

Diabetes mellitus (DM) is a metabolic disorder of the endocrine system, which is characterized by chronic postprandial hyperglycemia (high blood glucose). It either results from continued insulin resistance or pancreatic β cell malfunctioning or non-functioning [1]. The normal fasting and random blood glucose range is 70-99 mg/dL, and <140 mg/dL, respectively. DM is the 4th leading cause of mortality in the developed states. However, approximately 80% of diabetic patients are from developing and underdeveloped countries [2]. According to the International Diabetes Federation (IDF), 1 in 11 adults has DM [3]. Globally, Pakistan is the 10th major contributor to the global burden of diabetes and will attain the 8th position by 2045 [4]. DM has been cured by using different conventional methods such as allopathic medicines, herbal, and medical plants for several decades.

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Currently, DM is mainly treated by using anti-diabetic drugs, which keep the blood glucose concentration in normal range through supplementing the body with insulin, reduced insulin insensitivity, and increased insulin production lowering the absorption of glucose in the gastrointestinal tract, and/or uptake of glucose by the body tissues. However, regular consumption of glucose-lowering drugs may cause several side effects, such as digestive discomfort, dizziness, headache, idiosyncratic liver cell injury, lactic acidosis, permanent neurological deficit, severe hypoglycaemia, and even death. Furthermore, in developing countries, the majority of diabetic patients are unable to afford expensive drug treatment. In this context, the use of numerous herbal and medicinal plants for the management of diabetes is gaining popularity [5]. Complementary and alternative medicine (CAM) is now considered a more holistic approach for treating DM that combines the use of dietary supplements, herbs, and medicinal plants along with single conventional allopathic medicine [6]. A wide variety of medicinal plants and their respective products are used in various cultures for the treatment of DM. These medicinal plants are traditionally in use for centuries to treat several human health disorders including coronary heart disease, diabetes, cancer, and others [5,7]. World Health Organization has listed approximately 21000 medicinal plants, out of which 150 species are being used commercially as remedies against various health aliments [8]. Worldwide, approximately 30% of diabetic patients are using CAM while in Pakistan about 50% of patients are following CAM approaches just based on personal experiences and local communication. About 450 medicinal plants are experimentally verified to have anti-diabetic properties but only 109 of them are known for their complete mechanism of action [9]. Medicinal plants have numerous useful activities including antioxidant properties, improving glucose uptake and its utilization, insulin production, and manipulation of the carbohydrate metabolism through various mechanisms such as preventing damage, and restoring the integrity and functionality of β-cells. Seeds, pulp, and extracts of various medicinal plants and herbs and considered cost-effective and safe approaches [10, 11].

Momordica charantia is known with different names such as ku gua, balsam pear, karela, bitter gourd, or bitter melon. Its constituents are water (almost 90%), lipids (0.5%), protein (2%), carbohydrate (5%), fibre (2%) and ash (1%). Moreover, it contains various bioactive components such as phenolic, insulin-like molecules (polypeptides p or k, v-insulin, momordicin, charantin), alkaloid, steroid, and triterpene [12]. Roots, leaves, stems, and fruit of bitter melon, all parts are used to treat numerous health problems like digestive disorders, hyperlipidemia, menstrual problems, microbial infections, and hyperglycaemic [13].

Aloe barbadensis Miller is globally known as "*miracle plant"* or "silent healer" or the healing plant because of its splendid medicinal characteristics [14, 15]. Aloe vera is fully packed with more than 75 nutrients and 200 active components including lignin, anthraquinones, sugar, minerals, saponins, enzymes, vitamins, amino acids, and salicylic acid. Biologically active compounds extracted from aloe vera are informed to possess numerous medicinal properties such as promoting wound-, frostbite- and burn healing, in addition, have antibacterial, antifungal, anti-inflammatory, anti-inflammatory, antioxidant, antitumor, antitumor, antiviral, gastroprotective, hypoglycaemic, and laxative properties [16]. The anti-diabetic potential of aloe vera gel is linked with its crude polysaccharide components and inorganic minerals (i.e. copper, manganese, and vanadium). The anti-diabetic and anti-hyper cholesterolemia potential of bitter melon and aloe vera gel is well established in alloxan and streptozocin induced diabetics mice, rats, and rabbits, genetically diabetic mice, and in Type 2 diabetic humans [17, 18]. Both have a long history of use against DM in India, Pakistan, Africa, and Latin America [19]. The present study was undertaken to develop bitter melon seeds and aloe vera gel supplemented chicken nuggets for diabetic patients. The effectiveness of this dietary interaction was probed through a feeding trial in Sprague Dawley Rats.

Materials and Methods

Procurement of raw material

Bitter melon dried seeds and aloe vera were procured from Ayub Agricultural Research Institute (AARI), Faisalabad and Vegetable Research Area of Institute of Horticultural Sciences, University of Agriculture, Faisalabad respectively, during January 2017. All reagents were purchased from Merck (Merck KGa A, Darmstadt, Germany) and Sigma-Aldrich (Sigma Aldrich, Tokyo, Japan).

Preparation of bitter melon seeds and aloe vera gel powder

The dried bitter melon seeds were grounded into a fine powder of uniform size [20]. After harvesting, homogenous leaves of aloe vera were carefully chosen according to color, freshness, ripeness, and size followed by the washing with distilled water to remove dirt and other impurities. Aloe latex was removed and gel cut into small pieces [15, 21). The gel was placed in a dehydrator for 24 hours at 60°C. Dried flakes of gel were grounded into a fine powder [17]. The powdered seeds and gel were kept in airtight

containers at room temperature for further use in the study.

Table 1. Treatment plan used in the study

Treat	Bitter melon seeds powder (%)	Aloe vera gel powder (%)
T_0*	-	-
T_1	100	0
T_2	75	25
T_3	50	50
T_3	25	75
T_5	0	100

***T_0= Chicken nuggets without supplemented powder**

T_1= Chicken nuggets supplemented with 100% bitter melon seeds powder

T_2= Chicken nuggets supplemented with 75% bitter melon seeds powder and 25% aloe vera gel powder

T_3= Chicken nuggets supplemented with 50% bitter melon seeds powder and 50% aloe vera gel powder

T_4= Chicken nuggets supplemented with 25% bitter melon seeds powder and 75% aloe vera gel powder

T_5= Chicken nuggets supplemented with 100% aloe vera gel powder

Analysis of bitter melon seeds and aloe vera gel powder

Both samples were analysed in triplicate for proximate composition like moisture crude protein, fat, fibre, ash, and nitrogen-free extract (NFE) [22] and minerals like calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn) [23].

Preparation of functional chicken nuggets

Functional chicken nuggets were prepared by using the modified method of [24]. Bitter melon seeds and aloe vera gel powder were added in all formulations in different concentrations except control (Table 1.). All nuggets samples were packed in polyethylene zip bags and stored at freezing temperatures for further use in the study.

Analysis of functional chicken nuggets

Functional chicken nuggets samples were analyzed in triplicate for proximate and minerals composition and consumer acceptability.

Sensory evaluation of functional chicken nuggets

Sensory evaluation of nuggets was carried out for various characteristics like appearance, aroma, crispiness, palatability, taste, texture, and overall acceptability by a penal of judges using a 9- Point Hedonic Score system as described by [25].

Selection of best treatment

Based on consumer acceptability, the two best formulations were selected to find out their impact on blood glucose, HbA1c, insulin level, and lipid profile in the efficacy study.

Efficacy study

An efficacy study was conducted in Sprang Dawley Rats (2 months) to determine the anti-diabetic impact of bitter melon seeds and aloe vera gel powder. Healthy Sprang Dawley Rats weighing about 180-200 g were housed in an animal facility of the Faculty of Food Nutrition and Home Science, National Institute of Food Science and Technology, University of Agriculture, Faisalabad. The experiment was conducted according to the guideline of the Institutional Biosafety Committee (IBC). Rats were acclimatized for one week before the start of the experiment and a normal diet and water ad libitum were given. Afterward, they were fed, for 2 weeks, on the diet having 35% calories from sucrose [26]. 18 rats having fasting blood glucose level more than 120 mg/dL were selected for further use in the study and randomly divided into 3 groups i.e. Group I: Positive (+ve) control group fed on normal feed, Group II: Treated with supplemented diet (50% bitter melon seeds powder and 50% aloe vera gel powder) and Group III: Treated with supplemented diet (75% bitter melon seeds 25% aloe vera gel powder) up to 4 weeks. Blood samples were collected at the initiation, mid, and end of the study using a 23-gauge needle from the lateral saphenous vein, for biochemical analysis such as fasting blood glucose, HbA1c, lipid profile [19], and insulin level.

Statistical analysis

The data recorded for each parameter were analysed statistically using "Statistix 8.1" to determine the level of significance by a completely randomized design [27].

Results & Discussion

Analysis of bitter melon seeds and aloe vera gel powder

i. Proximate analysis

The proximate composition of bitter melon seed and aloe vera gel powder are given in Table 2. The results of proximate analysis of bitter melon seeds powder showed that moisture content was 6.5%, crude protein 9.25%, crude fat 17.50%, fiber 12.14%, ash 0.235%, and nitrogen-free extract (NFE) 54.54%. However, in aloe vera gel powder there was 4.62% moisture content, 12.05% crude protein, 4.03% fat, 12.7% fiber, 11.23% ash, and 54.99% NFE. The chemical and physical properties of food are directly altered by the different levels of fat and moisture. The proximate composition of bitter melon pericarp and seeds on different maturity stages revealed that seeds had

Table 2. Proximate composition (%) and mineral composition (mg/kg) of bitter melon seeds and aloe vera gel

Parameter	Bitter melon	Aloe vera
Moisture	6.5±0.03	4.62±0.41
Crude Protein	9.25±0.113	12.05±0.072
Crude Fat	17.50±0.16	4.03±0.18
Crude Fiber	12.14±0.26	12.7±0.6
Ash	0.235±0.001	11.23±0.15
NFE	54.54±0.22	54.99±0.16
Minerals (mg/kg)		
Sodium	15±1.03	75±2.06
Potassium	20±1.30	7±0.98
Calcium	25±1.16	70±1.01
Magnesium	80±1.26	86.25±2.45
Copper	20±1.10	212.5±1.26
Iron	144±1.22	10±1.89
Zinc	26±1.06	18±1.33

SD= ±
NFE= Nitrogen Free Extract

higher moisture content (7.3%) in the ripe stage compared to at (5.0%) fully mature stage. However, protein contents ranged from 14.2% to as higher as 28.2% and fat content ranged from 4.3% to 18.1% in mature seeds. A significant decrease in the protein and lipid concentration was noted as seeds progressed towards the mature phase [28, 29, 30]. However, nutritional analysis of leaf, seed, and pericarp of bitter melon fruit showed very low ash content approximately 0.03% whereas fiber was 17.5% [31]. Lower ash indicates lower mineral content in food material. However, aloe vera gel has higher ash content means it is more concentrated in minerals. Aloe vera gel was analysed for chemical and physical properties results revealed that protein contents ranged from 7% to 12% and fat content ranged from as little as 1% to 4% and carbohydrates ranges from 56 to 66% [32, 33]. The difference might be due to variation in variety that is analysed or probably due to the method used.

ii. Mineral analysis

Minerals analysis of bitter melon seed and aloe vera gel powder such as Na, K, Ca, Mg, Cu, Fe, and Zn of bitter melon seeds and aloe vera gel powder in milligram per kilogram (mg/Kg) are given in Table 2. Mineral's analysis showed that bitter melon seeds powder has a lower concentration (per kg) of Na, Ca, and Cu content (15 mg, 25 mg, and 20 mg) compared to aloe vera gel powder (75 mg, 70 mg, 212.5 mg) respectively. Whereas, K, Zn, and Fe were more concentrated in seeds powder (20 mg, 26mg, and 144) compare to gel powder (7 mg, 18 mg, and 10 mg) respectively. However, both powders have a similar concentration of Mg such as 80 mg in seed powder and 86 mg in gel powder.

Mineral analysis of bitter melon seeds on different maturity stages revealed that it has a much higher concentration (mg/g) of K, Mg, and Ca (8.6, 4.2, and 0.82 respectively) and slightly increase as maturity progresses. Whereas concentration (mg/kg) of Na ranges from 21 to 136mg, Cu content varies between 17.4 to 23mg, Fe ranges from 102 to 224.4 mg, and Zn content leads between 34.5 and 64 mg. However, development in maturity didn't significantly affect these micro minerals [29, 30]. The inorganic content of Aloe vera gel had been determined to access their impact on diabetes-related bio marks experimental animal models. Results revealed that gel had a comparable amount of Na (81 mg/kg), Ca (70.5 mg/kg) and Mg (78.5 mg/kg) whereas the concentration of K, Fe, and Zn (198.5 mg, 1.7g, and 189 mg) was quite higher. However, Cu content (75mg/kg) was lower [53]. The difference in mineral concentration might be due to variation in varieties, environments, and soil in which the plant was grown. Micro-minerals like Cu and Zn helps in lowering the glucose level in the body through attenuating insulin signalling and improving its' sensitivity in body cells abnormalities [34].

Analysis of functional chicken nuggets

i. Proximate analysis

Mean squares for the proximate composition of supplemented functional chicken nuggets are given in Table 3. Results revealed that bitter melon seeds and aloe vera gel powder exhibited a significant effect on moisture, crude protein, fat, fiber, and NFE but a non-significant effect on total ash.

Means squares for moisture content showed that maximum moisture content (54.70%) was found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3) followed by 54.69% in chicken nuggets supplemented with 100% aloe vera gel powder (T5) and 53.61% in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). However, the lowest moisture content (49.74%) was noted in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2) followed by 52.40% and 52.76% in chicken nuggets supplemented with 100% bitter melon seeds powder (T1) and in chicken nuggets without supplemented powder (T0), respectively. Overall, the moisture content of supplemented chicken was ranging from 49.74 to 54.70%. As the concentration of aloe vera gel increases the percent moisture also raised significantly, this may be because of the water holding capacity of gel powder [35].

Table 3. Means for proximate composition (%) and mineral composition (mg/kg) of supplemented functional nuggets

Parameters	Treatments					
	T_0*	T_1	T_2	T_3	T_4	T_5
Moisture	52.57±0.41a	52.40±0.44a	49.74±0.24b	54.70±0.28c	53.61±0.27c	54.69±0.25c
Fat	5.45±0.42a	5.67±0.17a	7.00±0.66b	7.16±0.17b	8.91±0.13b	6.61±0.13c
Protein	13.84±0.07a	14.19±0.17b	14.65±0.04c	14.21±0.04d	14.77±0.11d	15.32±0.05e
Fiber	5.00±0.1f	5.12±0.11e	5.23±0.13d	5.45±0.9c	5.75±0.10b	5.98±0.12a
Ash	1.12±0.06a	1.19±0.33de	1.20±0.07cd	1.31±0.02c	1.403±0.02e	1.47±0.02b
NFE	22.02±0.03f	21.43±0.22b	22.18±0.15a	17.17±0.29c	15.56±0.42e	15.93±0.23d
Sodium	227.3±2.52c	225.0±2.0c	232.0±2.65b	235.0±2.0b	235.3±1.53b	240.3±1.53a
Potassium	19.0±3.0a	19.0±1.0a	20.0±1.0a	19.7±1.5a	20.0±1.0a	19.67±1.5a
Calcium	21.0±2.0c	22.00±1.53bc	22.670±1.0bc	23.0±1.0bc	24.3±0.58ab	25.67±1.53a
Magnesium	77.17±1.53d	79.50±1.53c	80.583±1.0c	81.25±1.0c	85.25±1.0b	90.25±1.0a
Copper	28.42±1.5c	28.7±1.0c	31.5±1.0b	32.417±0.76b	34.5±1.0a	36.5±1.0a
Iron	29.33±1.53d	44.3±0.58a	34.33±0.58b	34.33±1.52b	32.7±2.08bc	30.7±1.53cd
Zinc	13.0±1.0b	16.67±1.53a	15.67±1.15a	15.33±0.58a	13.33±0.57b	13.33±0.58b
SD= ±	NFE= Nitrogen Free Extract					
	Means sharing same letters within a row are statistically non-significant					

Means squares for protein content of functional chicken nuggets showed that most protein content (15.32%) was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 14.77% in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4), 14.65% in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2), 14.21% in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3) and 14.19% in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Chicken nuggets without supplemented powder (T0) had shown the lowest protein content approximately 13.84% as it didn't have an additional contribution from bitter melon and aloe vera powder. Overall, the protein content of supplemented chicken was ranged from 13.84 to 15.32 %. Protein content increased with the increase in aloe vera gel powder in nuggets as it may be contributed by aloe vera gel powder.

Means squares for the fat content of treatments revealed that more fat content (8.91%) was found in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4), followed by 7.16%the chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). Whereas, lowest fat content (5.45%) was in chicken nuggets without supplemented powder (T0) followed by 5.67% in chicken nuggets supplemented with 100% bitter melon seeds powder (T1), 6.61% in chicken nuggets supplemented with 100% aloe vera gel powder (T5) and 7.00% in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2). Overall, the fat content of functional chicken nuggets was ranged from 5.45 to 8.91%. There was an increase in the fat content of supplemented chicken nuggets with the progressive increase in the level of aloe vera. The decrease in fat content in 100% supplemented aloe vera product, might be due to the development of the lipolytic activity of aloe vera.

The maximum fiber content of 5.98% was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 5.75% in the chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). However, the lowest fiber content (5.00%) was noted in chicken nuggets without supplemented powder (T0) followed by 5.12% in chicken nuggets supplemented with 100% bitter melon seed powder (T1). Supplementation of bitter melon seeds and aloe vera gel powder in nuggets gradually increased the fiber content according to the level of supplementation. Mainly aloe vera acted as the source of dietary fiber in functional chicken nuggets [36].

Means squares for ash content showed non-significant differences among the treatment. The ash content of chicken nuggets was ranged from 1.12% in chicken nuggets without supplemented powder (T0) to 1.47% in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). Supplemented chicken nuggets had slightly more ash content as compared to non-supplemented ones. Aloe vera gel powder contributed more to increasing ash content.

The most NFE content (22.18%) was noted in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2), followed by 21.43% in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). However, the lowest NFE content 15.56% was found in chicken nuggets supplemented with 25% bitter melon seeds and 75%

aloe vera gel powder (T4) followed by 15.93% in chicken nuggets supplemented with 100% aloe vera gel powder (T5). Overall, the NFE content of functional chicken nuggets was ranged from 15.56 to 22.18%.

ii. Mineral analysis

Means squares for the mineral composition of supplemented functional chicken nuggets are given in Table 3. Means for Na, Ca, Mg, Cu, Fe, and Zn concentration revealed significant differences among the treatments whereas, K content showed non-significant differences among the treatments.

The highest sodium content (240.33mg/kg) was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 235.33mg/kg in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). The lowest sodium content (225.0±2.0mg/kg) was noted in chicken nuggets supplemented with 100% bitter melon seeds powder (T1) followed by 227.33mg/kg in chicken nuggets without supplemented powder (T0). Overall, the sodium content of functional chicken nuggets was ranged from 240.33mg/kg to 225.00mg/kg. Sodium is also contributed by salt and other ingredients added in nuggets. Na gradually increased with the rise in aloe vera gel powder concentration as it has more Na compared to bitter melon seed powder.

The highest potassium content (20.00mg/kg) was found in T0, T2 and T4 followed by 19.67mg/kg in chicken nuggets supplemented with 100% aloe vera gel powder (T5). The lowest potassium content (19.00mg/kg) was noted in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3) and in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Overall, the potassium content of functional chicken nuggets was ranged from 20.00mg/kg to 19.00mg/kg. Both powders had a similar amount of K that's why a non-significant difference was present among the treatments.

The highest calcium content (25.67mg/kg) was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 24.33mg/kg in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). The lowest calcium content (21.00mg/kg) was noted in chicken nuggets without supplemented powder (T0) followed by 22.00mg/kg in chicken nuggets supplemented with 100% bitter melon seed powder (T1). Overall, the calcium content of functional chicken nuggets was ranged from 25.67mg/kg to 21.00mg/kg. Ca gradually increased with the rise in aloe vera gel powder concentration as it has more Ca compared to bitter melon seed powder.

Means for Mg content of functional chicken nuggets supplemented with bitter melon seeds and aloe vera gel powder revealed significant differences among the treatments. The highest magnesium content (90.25mg/kg) was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 85.25mg/kg in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). The lowest magnesium content (77.17mg/kg) was noted in chicken nuggets without supplemented powder (T0) followed by 79.50mg/kg in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Overall, the magnesium content of functional chicken nuggets was ranged from 90.25mg/kg to 77.17mg/kg. Mg gradually increased with the rise in aloe vera gel powder concentration as it has more Mg compared to bitter melon seed powder.

Means for Cu content of functional chicken nuggets supplemented with bitter melon seeds and aloe vera gel powder revealed significant differences among the treatments. The highest copper content (36.50mg/kg) was found in chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 34.50mg/kg in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). The lowest copper content (28.42mg/kg) was noted in chicken nuggets without supplemented powder (T0) followed by 28.7mg/kg in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Overall, the copper content of functional chicken nuggets was ranged from 36.50mg/kg to 28.42mg/kg. Cu gradually increased with the rise in aloe vera gel powder concentration as it has more Cu compared to bitter melon seed powder.

Means for Fe content of functional chicken nuggets supplemented with bitter melon seeds and aloe vera gel powder revealed significant differences among the treatments. The highest iron content (44.33mg/kg) was found in chicken nuggets supplemented with 100% bitter melon seeds powder (T1) followed by 34.33mg/kg in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2). The lowest iron content (29.33mg/kg) was noted in chicken nuggets without supplemented powder (T0) followed by 30.7mg/kg in chicken nuggets supplemented with 100% bitter melon seeds (T5). Overall, the iron content of functional chicken nuggets was ranged from 44.33mg/kg to 29.33mg/kg. Fe gradually increased with the rise in bitter melon seed powder concentration as it has more Fe compared to aloe vera gel powder.

Means for Zn content of functional chicken nuggets supplemented with bitter melon seeds and aloe vera gel powder revealed significant differences among the treatments. The highest zinc content (16.67mg/kg)

was found in chicken nuggets supplemented with 100% bitter melon seeds powder (T1) followed by 15.67mg/kg in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2). The lowest zinc content (13.00mg/kg) was noted in chicken nuggets without supplemented powder (T0) followed by 13.33mg/kg chicken nuggets supplemented with a higher concentration of aloe vera gel powder (T4 and T5). Overall, the zinc content of functional chicken nuggets was ranged from 16.67mg/kg to 13.00mg/kg. Zn gradually increased with the rise in bitter melon seed powder concentration as it has more Zn compared to aloe vera gel powder.

Sensory Evaluation

The means for appearance, aroma, texture, taste, crispiness, palatability, and overall acceptability revealed significant differences among the treatments as well as in the storage intervals (Table 4.). Appearance is the first sensory characteristic perceived by the consumer at a glance. It is the first score given to the food commodity. The mean values of appearance of different treatments showed that the highest score (8.05) was found in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2), followed by 8.03 in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). The lowest score was 6.55 in the chicken nuggets supplemented with 100% aloe vera gel powder (T5) preceded by 7.23 found in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). T2 showed a better appearance due to its light brown color while T5 showed greenish-brown color due to the highest level of aloe vera gel powder supplementation.

The mean values of aroma showed that the highest score 7.75 was found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3) followed by 7.33 in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2). The lowest 5.57 score in the chicken nuggets supplemented with 100% aloe vera gel powder (T5) was preceded by 6.87 found in chicken nuggets supplemented with (T1). The treatment T3 showed a strong aroma due to the presence of bitter melon and aloe vera gel powder in equal proportions. There was a decreasing trend in the aroma as the concentration of bitter melon seeds and aloe vera gel powder increased. The very light aroma was observed in T1 as it has no aloe vera powder supplementation,

Texture properties can affect the perceived flavor [37]. The mean values of the texture of different treatments showed that the highest score 8.13 was found in chicken nuggets without supplemented powder (T0) followed by 7.60 found in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2) due to its crispy texture. The lowest 5.87 was scored to chicken nuggets supplemented with 100% aloe vera gel powder (T5) followed by 6.80 found in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4) due to the presence of fiber-containing bitter melon seeds and aloe vera gel powder. The mean values of the texture of chicken nuggets at 0- and 30-day intervals were 7.13 and 6.65. From the mean values for storage, it was revealed that the texture of chicken nuggets decreased during storage. The decrease in quality score for texture was due to absorption of moisture that has a negative impact on texture. Texture scores followed a significant decreasing trend throughout the storage period when aloe vera was added to chicken nuggets [36].

The mean values of the taste of different treatments showed that the highest score (7.57) was found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). The lowest 5.0 was scored to chicken nuggets supplemented with 100% aloe vera gel powder (T5) preceded by 6.80 found in chicken nuggets supplemented with 25% bitter melon seeds and 75% aloe vera gel powder (T4). The chicken nuggets with the highest percentage of aloe vera gel showed a bitter taste while the treatment (T3) in which both aloe vera gel powder and bitter melon seeds were supplemented in equal proportions showed the best taste among other treatments.

The crispiness of different treatments of functional chicken nuggets differed significantly among various treatments of functional chicken nuggets according to the analysis of variance mentioned. Mean values for the crispiness of functional chicken nuggets range from 5.18 to 7.52. The mean values of crispiness of different treatments showed that the highest score of 7.52 was found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). The lowest 5.18 was scored to chicken nuggets supplemented with 100% aloe vera gel powder (T5) preceded by 6.70 found in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Chicken nuggets with the highest aloe vera concentration have the lowest crispiness as the fibrous powder of gel soak the moisture and become soggy and soft and lose its crispiness compare to other formulations.

Table 4. Means for sensory attributes of supplemented functional nuggets

Attributes	Treatments					
	T_0*	T_1	T_2	T_3	T_4	T_5
Appearance	7.00±2.17c	7.28±0.02b	8.05±0.02a	8.03±0.00a	7.23±0.00bc	6.55±0.02d
Aroma	6.75±0.05c	6.87±0.05c	7.33 ±0.05b	7.75 ± 0.05a	6.92±0.05c	5.57±0.05d
Texture	6.72±2.00b	6.80±0.05b	7.60±0.00a	7.55±0.02a	6.80±0.66b	5.87±0.66c
Taste	6.97±2.31b	7.05±0.02b	7.43±0.00a	7.57±0.00a	6.88±0.02b	5.00±0.05c
Crispiness	6.83±1.98c	6.70±0.00c	7.28±0.02ab	7.52±0.02a	6.87±0.00bc	5.18±0.02d
Palatability	7.85±0.92a	6.68±0.02c	7.63±0.00ab	7.70± 0.00a	7.05±0.02bs	5.85±0.07d
Overall Acceptability	7.22±1.65b	6.88±0.01c	7.52±0.01a	7.66±0.00a	6.92±0.00c	5.63±0.01d
SD= ±	*Means sharing the same letters within a row are statistically non-significant*					

The mean values of palatability of different treatments showed that the highest score of 7.85 was found in chicken nuggets without supplemented powder (T0) followed by 7.70 found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3). The lowest 5.85 was scored to chicken nuggets supplemented with 100% aloe vera gel powder (T5) preceded by 6.68 found in chicken nuggets supplemented with 100% bitter melon seeds powder (T1). Chicken nuggets without supplementation are more palatable, while nuggets with the highest concentration of aloe vera gel powder are least palatable as compared to other treatments.

Mean values of overall acceptability of different treatments showed that the highest acceptability was 7.66 found in chicken nuggets supplemented with 50% bitter melon seeds and 50% aloe vera gel powder (T3) due to best appearance, aroma, crispiness, taste, and texture followed by 7.22 found in chicken nuggets without supplemented powder (T0) due to better appearance, aroma, texture, crispiness, taste and palatability and 7.52 in chicken nuggets supplemented with 75% bitter melon seeds and 25% aloe vera gel powder (T2) due to good appearance, aroma, texture, crispiness, taste, and palatability compared to other formulations. Lowest acceptability of chicken nuggets supplemented with 100% aloe vera gel powder (T5) due to bitter taste and tough texture.

Selection of best treatments

Based on the overall acceptability of T2 (75% bitter melon seeds and 25% aloe vera gel powder) and T3 (50% bitter melon seeds and 50% aloe vera gel powder), these 2 combinations were selected to check their anti-diabetic potential in the animal model.

Efficacy study

Fasting blood glucose

Means for fasting blood glucose revealed that there was a significant difference among all groups (Table 5.) and the time interval also showed a significant reduction (Table 6.) Maximum fasting blood glucose concentration (148.33mg/dL) was observed in rats fed on a normal diet containing no supplemented powder (G0) followed by 109.67mg/dL and 106.28mg/dL in groups fed on 75% bitter melon seeds and 25% aloe vera gel powder (G2) and 50% bitter melon seeds and 50% aloe vera gel powder (G1), respectively. G1 showed a 28.39% reduction whereas G2 showed a 26.06% reduction compared to the control group (Figure 1). At the start of the experiment, the mean values of glucose level were 143.33mg/dL which gradually decreased to 111.39mg/dL and 109.56mg/dL by consuming supplemented diet. Over the period of 30-day fasting blood glucose decreased by 23.56% (Figure 2). It is obvious from the present findings, that consumption of bitter melon seeds and aloe vera gel powder in both combinations may help to control hyperglycaemic. Nkambo et al. [38] also revealed that methanolic extract of bitter melon at a dose of 125mg/kg had the potential to reduce fasting blood glucose. However, anthraquinones such as aloe-emodin, emodin, and chrysophanol, from aloe vera's gel at the dose of 10 µM were effective to inhibit the formation of advanced glycation end products nearly 66%. This shows that these compounds are directly or indirectly involved in mechanisms that are responsible for the maintenance of the blood glucose level [39].

Insulin

Means for serum insulin showed that there was a significant difference among all groups (Table 5.) and time intervals also showed a significant effect (Table 6.). Rats fed on 75% bitter melon seeds and 25% aloe vera gel powder (G2) showed improvement in the concentration of insulin by 94.15% (Figure 1) over 30 days whereas G1 showed a reduction from 3.33µIU/mL to 1.93µIU/mL in insulin concentration during first 15 days then starts to improve over the time up to 2.38µIU/mL. However, insulin concentration decreased by 35.64% over 30 days (Figure 2). It is evident from the present findings that consumption of bitter melon seeds and aloe vera gel powder was effective in maintaining as well as improving insulin levels. Certain bioactive compounds in bitter melon and aloe vera gel may have the ability to release insulin from β cells by stimulation

of them or may through involved in the process that is responsible for insulin production or through the revitalization of the β cells [41]. Insulin-like molecules such as p-insulin and v-insulin are mainly responsible for insulin-mimetic activity. Bitter melon consumption controls glucose level mainly through removal of the glucose from the blood, not by increasing insulin in the blood. Its insulin-like bioactive components are mainly responsible for this [41]. Consumption of bitter melon fruit juice significantly increases the number of β cells of the pancreas in the diabetic animal model [42].

HbA1c

Mean value for the effect of bitter melon seeds and aloe vera gel powder in combination on serum HbA1c of diabetic Sprague Dawley Rats revealed that maximum HbA1c concentration (5.77%) was found in control group rats fed on normal with no supplemented powder followed by 5.23% and 4.98% in groups fed on 75% bitter melon seeds and 25% aloe vera gel powder (G2) and 50% bitter melon seeds and 50% aloe vera gel powder (G1) respectively. At the start of the trial, the mean values of HbA1c were 5.50% which gradually decreased to 5.23% by consuming bitter melon seeds and aloe vera gel powder. A nearly 4.45% reduction in HbA1c was observed. It is obvious from the present findings, that consumption of bitter melon seeds and aloe vera gel in combination may help to control HbA1c in diabetics' individuals. The level of (HbA1c) in diabetics is directly linked to the concentration of glucose in the blood. With the improvement in glycaemic control and glucose tolerance, HbA1c also decreases. Powder and aqueous extract of bitter melon fruit are effective in lowering HbA1c significantly [43, 44]. Results of the present study were closely related to the findings of Devaraj et al., 2013; Alinejad-Mofrad et al., 2015, as they showed that the supplementation of aloe vera gel was responsible for the reduction of HbA1c in type II diabetes patients but showed no significant effect in pre-diabetics.

Table 5. Means for the effect of various treatments on fasting blood glucose, insulin, HbA1c and lipid profile triglycerides

Parameters	Groups		
	G_0*	G_1	G_2
Glucose (mg/dL)	148.33±13.59 a	106.28±32.00b	109.67±33.74b
Insulin (µIU/mL)	1.88±1.163c	2.55±0.72b	3.65±0.94 a
HbA1c (%)	5.77±0.143a	4.98±0.39c	5.23±0.188b
Cholesterol	167.61± 6.18a	125.44±33.57c	133.61±27.78b
HDL	33.66± 4.07b	32.00±0.93b	37.94±3.95a
LDL	107.89± 2.59a	69.83±33.30b	68.28±34.45b
Triglycerides	150.22±10.52 a	140.06±3.38b	137.33b±16.77b

G_0= Diabetic control

G_1= 50% bitter melon seeds powder and 50% aloe vera gel powder

G_2= 75% bitter melon seeds powder and 25% aloe vera gel powder

Cholesterol

Mean value for the effect of bitter melon seeds and aloe vera gel powder in combination on serum cholesterol revealed that maximum cholesterol concentration (167.61mg/dL) was found in control and 125.44mg/dL in groups fed on 50% bitter melon seeds and 50% aloe vera gel powder (G1) and 75% bitter melon seeds and 25% aloe vera gel powder (G2) respectively. At the start of the study, mean values of cholesterol were 161.94mg/dL which gradually decreased to 138.89mg/dL and 125.83mg/dL by consuming bitter melon seeds and aloe vera gel powder. The rats consuming 50% of bitter melon seeds and 50% aloe vera gel showed a maximum cholesterol reduction of 25.16% followed by rats consuming 75% bitter melon seeds and 25% aloe vera gel powder induced 20.29% reduction. However, over 30 days there was 22.29% reduction was observed in cholesterol levels. It is obvious from the present findings, that consumption of bitter melon seeds and aloe vera gel in combination may help to control serum cholesterol in diabetics individuals. Results of the present study were closely related to the findings of Nasiff et al. [47], results showed that patients with hyper-lipidaemia that were receiving a daily dose of juice of aloe vera showed a significant reduction in total serum cholesterol decreased by 15.4%. It was found that oral intake of the aloe vera gel significantly

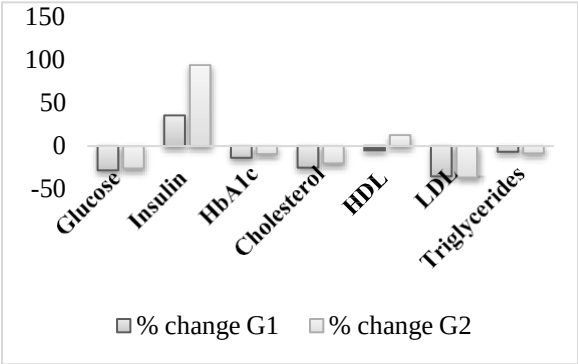

Figure 1. Percent change of G1 and G2 in fasting blood glucose, insulin, HbA1c and lipid profile.

lowered the total cholesterol by 61% [48]. Bitter melon had also been reported to decrease serum cholesterol levels in animal models significantly [49].

Table 6. Means ± SD for the effect of study intervals on fasting blood glucose, insulin, HbA1c and lipid profile

Parameters	Study interval (Days)		
	S_0	S_{15}	S_{30}
Glucose (mg/dL)	143.33±7.97a	111.39±32.33b	109.56±43.28b
Insulin (µIU/mL)	3.23±0.10a	2.08±0.94c	2.76±1.80b
HbA1c (%)	5.50±0.11a	5.23±0.47b	5.25b ±0.62
Cholesterol	161.94±1.18a	138.89±27.97b	125.83±40.63c
HDL	35.72±2.67a	32.72±2.46b	35.16±6.35a
LDL	106.39±1.07a	74.94±28.78b	64.67±39.57c
Triglycerides	142.06±7.62a	145.17±5.08a	140.39±20.98a

The Means sharing the same letters within a row are statistically non-significant

HDL

Mean value for the effect of bitter melon seeds and aloe vera gel powder in combination on high-density lipoprotein cholesterol (HDL-Cholesterol) of diabetic Sprague Dawley Rats revealed that maximum HDL-Cholesterol concentration (37.94mg/dL) was found in rats fed on 75% bitter melon seeds and 25% aloe vera gel powder (G2) followed by 32mg/dL in groups fed 50% bitter melon seeds and 50% aloe vera gel powder (G1).

At the start trail, mean values of HDL-Cholesterol were 35.72mg/dL which decreased to 32.72mg/dL in further 15 days and then raised to 35.16mg/dL by consuming supplemental powders of bitter melon seeds and aloe vera gel. The group consuming 75% bitter melon seeds and 25% aloe vera gel powder (G2) supplemented diet showed an increase in HDL-Cholesterol by 12.71% during the study. The group consuming 50% bitter melon seeds and 50% aloe vera gel powder (G1) supplemented diet showed little or no reduction in HDL-Cholesterol as compared to the control group which showed 16.96% reduction. It is evident from the present findings that consumption of 75% bitter melon seeds and 25% aloe vera gel powder is effective in improving HDL-Cholesterol and bitter melon seeds and aloe vera gel powder in combination with 50% is effective in maintaining the HDL-

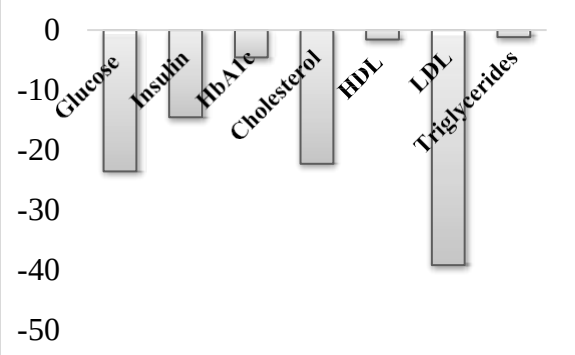

Figure 2. Percent change during the study in fasting blood Glucose, insulin, HbA1c, and lipid profile.

results of the present study were closely related to the findings of Dixit and Joshi [48] whose results showed that orally administered aloe vera gel potentially raised the HDL in diabetic patients. Bioactive compounds in bitter melon potentially increased the expression of apolipoprotein A which was a major part of HDL-Cholesterol and help to improve the concentration of it in serum [50].

LDL

Mean value for the effect of bitter melon seeds and aloe vera gel powder in combination on low-density lipoprotein of diabetic Sprague Dawley Rats showed that maximum LDL-Cholesterol concentration (107.89mg/dL) was found in diabetic rats fed on normal feed with no supplemented bitter melon and aloe vera gel powder followed by 69.83mg/dL and 68.28mg/dL in groups fed on 50% bitter melon seeds and 50% aloe vera gel powder (G1) and 75% bitter melon seeds powder and 25% aloe vera gel powder (G2) respectively. At the start, the mean values of LDL-Cholesterol level were 106.39mg/dL which gradually decreased to 74.94mg/dL and 64.67mg/dL by consuming supplemented diet. The groups consuming a supplemented diet with bitter melon seeds and aloe vera gel powder showed a reduction in LDL-Cholesterol about 39.21%. The group that was consuming a normal diet with no supplementation (G0) showed raised levels of LDL-Cholesterol during the study. It is obvious from the present findings, that consumption of a supplemented diet with bitter melon seeds and aloe vera gel powder in combination may help to control LDL-Cholesterol levels for diabetic individuals. Results of the present study were closely related to the findings of Nasiff et al. [47] showed that patients with hyper-lipidaemia that were receiving a daily dose of juice of aloe vera showed a significant reduction in LDL-Cholesterol by 18.9%. Bioactive components in bitter melon were responsible for improving serum lipid concentration as they had the potential to reduce the secretion of apolipoprotein B and apolipoprotein C- III expressions, both found in LDL-Cholesterol and VLDL-Cholesterol, hence they

are responsible for controlling their concentration in blood [50, 51].

Triglycerides

Mean value for the effect of bitter melon seeds and aloe vera gel powder in combination on triglycerides of diabetic Sprague Dawley Rats revealed that maximum triglycerides concentration (150.22mg/dL) was found in rats fed on a normal diet containing no supplemental bitter melon seeds or aloe vera gel powder followed by 140.06mg/dL and 137.33mg/dL in groups fed on 50% bitter melon seeds and 50% aloe vera gel powder (G1) and 75% bitter melon seeds and 25% aloe vera gel powder (G2), respectively. At the start of the experiment, the mean values of triglycerides were 142.06mg/dL which gradually increased to 145.17mg/dL and then decreased to 140.39mg/dL by consuming supplemented diet. The group (G2), consuming 75% bitter melon seeds powder and 25% aloe vera gel powder supplemented diet showed maximum triglycerides reduction (8.58%) followed by slight or almost significant decrease in triglycerides levels in blood-consuming 50% bitter melon seeds and 50% aloe vera gel powder supplemented diet (6.76). The group consuming a normal diet with no bitter melon seeds and aloe vera gel powder supplementation showed a significant increase in triglycerides by 1.17%. It is obvious from the present findings, that consumption of bitter melon seeds and aloe vera gel powder in a 50% combination may help to control blood triglycerides in diabetic patients. Results of the present study were closely related to the findings of Nasiff et al. [47] which showed a 31.9% reduction in triglycerides level of patients with hyper-lipidaemia that were receiving a daily dose of juice of aloe vera. Bitter melon juice and its alcoholic extract had a significant potential to lower the cholesterol and triglyceride levels in a diabetic animal model [52].

Conclusions

The groups consuming bitter melon seeds and aloe vera gel powder supplemented diet showed a significant reduction in blood glucose (41%), HbA1c (13.5%), and LDL-Cholesterol (60%). Whereas there was a rise in HDL-Cholesterol (15.69%) and insulin level (33.8%). However, diabetic control showed an increase in glucose level (17%), HbA1c level (4.82%), cholesterol level, triglycerides (13.1%), and ALT level (57.83%).

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Conflict of interest

The authors declare that no conflict of interest exists.

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