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Development of Novel Fermented Almond Milk Tea and It's Evaluation as Antidiabetic Drink

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Authors' contributions

This work was carried out under the supervision of author PS. Authors DK and SV finished the work. This paper was written by author AK. All authors read and approved the final manuscript.

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ABSTRACT

Background: It is well known that almond and tea is best known to prevent Diabetes mellitus due to its abundant source of polyphenols. Also, probiotics also have been used in the treatment of Diabetes. This study is focused on the combined effect of all these three ingredients through the process of fermentation.
Objective: The aim of this present study is to develop, analyse sensory parameters in human volunteers for optimisation and evaluate the antidiabetic efficiency of Fermented Almond milk tea (FAMT) both in vitro and in vivo analysis.
Study Design: Development of FAMT→Optimisation of FAMT based on sensory analysis from 25 human participants→ *In vitro* antidiabetic analysis of FAMT extract → Animal studies.
Place and Duration: The research work was conducted during November, 2019 to March, 2020 at the Department of Biotechnology, Sri Venkateswara College of Engineering, Post Bag No.1, Pennalur, Sriperumbudur Tk, Kancheepuram Dt, TN-602117, India.

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Materials and Methods: FAMT was prepared by optimisation of different formulation based on sensory analysis recorded from 25 healthy human volunteers. The FAMT extract was prepared and was used for the *in vitro* analysis and phytochemical screening. The animal study was performed with 30 Albino Wistar rats which were divided into 5 groups under preventive regimen. Group I was healthy normoglycemic control group. Group II served as positive control. Group III received metformin (350 mg/kg bw, p.o) for 28 days. Group IV received 5% Fermented almond milk for 28 days. Group V received 5% FAMT for 28thday. All groups except Group I received single dose of STZ (50 mg/kg bw, i.p) on the 29th day for the induction of Diabetes mellitus. After 7 days from induction, animals were anaesthetized and blood was drawn for the evaluation of plasma glucose and serum TG, cholesterol & insulin.

Results: It was observed that FAMT (8:2) was favoured by the participants more than other formulations. FAMT was found to contain Saponins, flavonoids and phenol. The total poly phenol of FAMT (373 ± 3.0 µg/ml) was high than Fermented almond milk (232.5 ± 2.50 µg/ml). The DPPH scavenging, α-amylase and α-glucosidase inhibiting percentage of FAMT (59 ± 4%,52 ± 3%, 50 ± 4% respectively) was high when compared to fermented almond milk (32 ± 2%,34 ± 2% and 45 ± 2% respectively). From animal studies it was significantly observed that plasma glucose ($P<0.0001$) was reduced, serum insulin ($P<0.001$) was increased, serum TG ($P<0.0.001$) and cholesterol ($P<0.01$) were reduced when compared to Positive control Group- II.

Conclusion: Thus, FAMT was proved to act as a prophylactic anti-diabetic drink and was more potent than normal fermented almond milk.

Keywords: FAMT; almond; tea; probiotics; antidiabetic drink.

ABBREVIATIONS

DNS : Dinitro salicylic acid
MPa : Mega Pascal
rpm : Rotation per minute
PBS : Phosphate-buffered saline
FAMT : Fermented Almond Milk Tea
DPPH : 2,2-diphenyl-1-picrylhydrazyl
nm : Nano meter
mL : Milli litter
ABS : Absorbance
µL : Micro litter
mg : Milli gram
STZ : Streptozotocin
p.o : Per os
i.p : Intra peritoneal

1. INTRODUCTION

Type 2 Diabetes mellitus is known to cause unfavourable effects like endothelial dysfunction and cardiovascular disease which in turn affects appx 425 million people world-wide. It is recommended to use screening tools to identify individuals who are the risk of Diabetes mellitus in order to carry out preventive measures [1]. Poor glycaemic control may be reflected by both the failure of diabetes self- management by patients and also by following inadequate control strategies. In general, failure in self-management depends on food culture which always has influence in leading a healthy life. Clinicians may further influence the patient's perception through effective communication skills and by having a well-integrated health care system [2]

Discussing about the disease targeted in this research paper, Diabetes Mellitus is a metabolic disorder that is characterised by increase in blood glucose level. It is mainly because of the insufficient secretion of insulin in the body. Due to irregular management of glucose metabolism in the body, these abnormalities gets reported in the body [3]. It was reported that more than 400 traditional herbal treatments were recorded for treating Diabetes mellitus and among them only a few of them have received medical attention and assessment for analysing its efficacy. Various hypoglycaemic phytocompounds have been identified by scientists that confirmed the antidiabetic effect on animal models and non-insulin dependent diabetic patients [4].

Although there are many phytoextracts being analysed for their presence of antidiabetic activity, interest on working and analysing the properties of fermented foods are peaking now a days. Subjecting raw food materials to microbial conversion produces fermented foods. Fermentation leads to an increase in the consistency and health-promoting ability of the foods (nutritional and organoleptic) as well as extending the shelf life. Depending on the geographical area, climate conditions, and source of availability, the usage and types of

Fermented foods differ among individuals. Fermented Foods are stated to prevent and control metabolic problems, cardiovascular diseases, cognitive development, immunity enhancement and many such health benefits. A drastic increase in consumption of fermented foods can be seen due to an increase in awareness in its health benefits. The process of fermentation and presence of active microbes in the procedure play an important role in the functional properties of Fermented Foods. The therapeutic properties of Fermented foods such as antiinflamatory, anticancer, antioxidant properties, improved gastro-intestinal health and reduced presence of metabolic disorders have been documented in several *in vitro* and *in vivo* studies [5].

It was reported that in an experiment of glucose tolerance, researchers observed that green tea facilitated glucose metabolism in healthy humans who volunteered. In genetically modified diabetic mice and streptozotocin induced mice, green tea reduced blood glucose levels [6]. It was noted that probiotic Lactobacillus exerts antidiabetic and antiinflamatory effects by enhancing glucose tolerance, hyperglycaemia, hyperinsulinemia, dyslepsia, oxidative stress and immune-regulatory properties and also protects complications caused by type 2 diabetes in high fructose-fed type 2 diabetes rats [7]. In today's growing food demand, fermented almond milk is viewed to be a healthier choice due to its combined benefits of almond milk and probiotics where both act as antidiabetic agents. The fermentation of almond milk using the probiotic Lactobacillus and Streptococcus species was studied by improvising various factors to ensure a high probiotic strain in the finished products [8]. The improvised protocol was used in this research work.

This study aims in combinational effect of almond, tea and probiotics in preventing diabetes mellitus. Fermentation technology was used in this study to combine the ingredients and formulate a novel diabetes preventing drink.

2. METHODOLOGY

2.1 Materials and Chemicals Used

Almond and green tea were procured from the local super market at Chennai. Metformin tablet was purchased from local medical shop in Chennai. Streptozotocin, alpha amylase, alpha glucosidase, DNS reagent and other basic level chemical used in this research work was purchased from Sigma-Aldrich Ltd. The probiotic organism used in this study was *Lactobacillus casei* (NCIM 2125).

2.2 Production Process Making of FAMT

2.2.1 Preparation of almond milk

The almond milk was procured and thoroughly soaked and grinded to extract the almond milk. Later, the almond milk was exposed to High pressure homogenisation treatment which was carried out at 172 MPa and pasteurised at 121°C for 15 mins leading to destruction of all vegetative cells [8].

2.2.2 Tea extract

Once the tea leaves were procured and washed, the green tea was then boiled at 120°C to get the extract.

2.2.3 Inoculum preparation

The strains were centrifuged at 9000 rpm for 10 min to separate cell biomass from the broth. Once done, the bacteria were resuspended in PBS 1x Buffer right away until it reaches 1.5 x 10^8 colony forming units (CFU) per mL (using 0.5 McFarland standard) [8].

2.2.4 Preparation of FAMT

Once the bacterial suspension was produced, 3 ml of the suspension is used per 100 ml of the processed almond milk for inoculation. The incubation was carried out at 40°C which in this case is the optimal temperature of the mixed culture. The end of the fermentation process was when the sample reached a pH of ≈4.6. The samples were then cooled at 4°C and stored until analysis is carried out [8].

2.3 Sensory Analysis

Four different formulations of FAMT (based on Almond milk: tea) was prepared and considered for Sensory analysis evaluation in 25 healthy volunteers. Fermented almond milk, FAMT (5:5), FAMT (8:2) and FAMT (4:6) were chosen for evaluation from 25 volunteers. The parameters that were considered were Flavour, Aroma, Taste and Overall acceptability. The scores were marked according to the method of Ranganna et al. [9] on a 9-point hedonic scale (Indications: 9 =

like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much and 1 = dislike extremely). The results were expressed in mean ± SEM.

2.4 Preparation of FAMT Extract

10ml of the prepared fermented almond milk tea was acidified to pH 4.0 with HCl (1 M) followed by heating it in water bath at 45 °C for 10 min and then subjected to centrifugation at 3,500 rpm for 10 min at 4°C. The supernatant was harvested and the pH is adjusted to 7.0 using NaOH (3 M) and re-centrifuged at 3,500 rpm for 10 min at 4°C for more precipitation of proteins and salts. Then the supernatants were harvested and stored at 4°C. This final product is called as FAMT extract which is used for the in vitro analysis assays [10].

2.5 Phytochemical Screening

Test for phenols - 5% ferric chloride was added to 1ml of FAMT extract and the colour change was observed [11].

Test for flavanoids -2% NaOH was added to 1ml of FAMT extract. The colour change and the disappearance of colour upon the addition of HCl was observed [12].

Test for Saponins – 5ml distilled water was added to 1ml FAMT before it was shaken vigorously for 15 mins and formation of froth was checked. To the froth a drop of olive oil was added and shaken vigorously and checked for appearance of foam [12].

2.6 Total Polyphenol Estimation

The polyphenolic content was estimated using *Folin-Ciocalteu* assay, by employing gallic acid standards. 5 ml *Folin-Ciocalteu* reagent (1:10 dilution) was added to the 1ml of FAMT extract or standard (Gallic acid), mixed and incubated for 5 min at room temperature before the addition of 5ml of 7.5% Na_2CO_3. The mixture was incubated for 2 H before absorbance readings was taken at 765 nm [13].

2.7 *In vitro* Antidiabetic Analysis of FAMT

2.7.1 DPPH scavenging activity

The free radical scavenging activity of the prepared almond milk tea was assessed with ascorbic acid as reference compound. 3.9 ml of DPPH solution (2.4 mg in 100 mL of methanol) was then added to 0.1 ml varying concentrations of the FAMT extract. The mixture was vigorously shaken and subjected to incubation for 30 mins in the dark. The absorbance was measured at 515 nm using spectrophotometer. The percentage of radical scavenging activity was calculated by noticing the decrease in the colour of the reagent [14].

2.7.2 Alpha amylase inhibition activity

Based on the spectrophotometric assay, *In vitro* alpha-amylase inhibition assay was carried out. An acarbose compound is used as a reference compound [15]. The revised technique of DNSA was performed in which 1ml FAMT extract was added with 1 mL of 1 Unit/mL alpha-amylase solution at room temperature for 30 minutes, followed by adding 1 mL (1% w/v) starch solution, and was then further incubated for 30 minutes at 37º C. Finally, with the addition of 1 mL of DNS reagent and the contents in a water bath for 5 minutes, the reaction was halted. One blank, without FAMT extract was prepared and replaced by equal proportions of buffer (20 mM sodium phosphate buffer with 6.7 mM sodium chloride, pH 6.9 at 20ºC). The absorbance was recorded at 540 nm. To determine the % inhibition of alpha amylase, the following formula was used.

% Inhibition = [1- (ABS sample / ABS control)] x 100

2.7.3 Alpha glucosidase inhibition activity

The assay was performed as described by Apostolidis et al. [16]. 100 µL of α-Glucosidase solution (1 U/mL) in 100 mM of phosphate buffer (pH 6.9) was added to 50µL of FAMT extract and incubated at 25ºC for 10 minutes. Then, in a 100 mM phosphate buffer solution (pH 6.9), 50 µL of 5 mM p-nitrophenyl d-glucopyranoside solution was added. The mixtures were incubated for 5 minutes at 25ºC and the absorbance readings in the spectrophotometer were taken at 405 nm. The inhibitory activity was recorded as a percentage inhibition which is stated as follows:

% Inhibition = [1- (ABS sample / ABS control)] x 100

2.8 *In vivo* Studies

2.8.1 Animal maintenance, grouping and diabetes induction

The experimental study was conducted at Sri Venkateswara College of Engineering,

Institutional Animal House, with the protocol approved by IAEC SVCE: Protocol-1/Phase-2/2019 (Ref.: 10/IAEC/Dated 23rd Dec, 2019). The study was conducted on 30 matured *Wistar strain* male albino rats (TANUVAS, Tamil Nadu, India). Prior to the experiment, the animals were acclimated for a period of 15 days in our laboratory conditions. Rats were housed in cages, at an ambient temperature of 25±2° C with 12 h light: 12 h dark cycle. Rats had free access to standard food and water *ad libitum*. The Principles of Laboratory Animal Care (CPCSEA) is followed throughout the duration of experiment and instruction given by our institutional ethical committee was followed regarding injection and other treatment of the experiment. Animals were grouped into 6 groups each containing six animals as per the Table 1. Diabetes mellitus was induced by single dose of STZ (50 mg/kg body weight, i.p) in 0.1 M citrate buffer (pH 4.5).

2.8.2 Serum bio-chemical study

After 7 days of STZ induction, blood was collected from the animals by cardiac puncture method after anaesthetising with Pentobarbitone sodium (35 mg/kg body weight, i.p.). Immediately, serum and plasma were separated for analysis of plasma glucose and serum triglyceride (TG), insulin and cholesterol.

2.9 Statistical Analysis

The results were expressed as mean ± SEM. Statistical significance between means was analyzed by One Way Analysis of Variance followed by "Dunnett's test" where $P<0.05$ was considered less significant, $P<0.01$ was considered as significant, $0.01<P<0.001$ was considered as moderately significant, $P<0.0001$ was considered statistically highly significant.

3. RESULTS AND DISCUSSION

3.1 Preparation of FAMT

The combined methodology followed by [8,6], lead to the formulation of FAMT as in Fig. 1. The FAMT was found to be stable for two days under 4° C. While over period of storage lead to phase separation. This was also in accordance with Bernat et al. [8].

3.2 Sensory Analysis

The scores for the sensory parameters of FAMT recorded from 25 heathy human volunteers were tabulated as in Table 2.

Statistical significance between means was analysed by One Way Analysis of Variance followed by "Bonferroni's test" for comparing FAMT with fermented almond milk and it was found that the statistics showed no significance. Thus, by visualising scores allotted by the participants, it was concluded that FAMT (8:2) formulation was with better flavour, taste and overall acceptability than the other formulation. Since the tea content was more in FAMT (4:6) when compared to other formulations, tea aroma dominated and was favoured by the participants more. However, since this study focusses more on developing it as the marketable product, considering the other parameters, FAMT (8:2) formulation was selected for further studies based on the scores allotted by the participants.

3.3 Phytochemical Screening

The prepared FAMT in its stable condition was analysed for preliminary phytocomponents like phenol, flavonoid and saponin Table 3 and Fig. 2. It was found that generally these compounds possess anti-diabetic property. Phenols were reported to give protection to beta cells of pancreas against glucose toxicity. Flavonoids were also reported to inhibit hyperglycaemic effect. They inhibit oxidative stress, nitric oxide stress and inflammatory cytokines. These properties induce the body to release insulin [17]. Saponins, another anti-diabetic agent, was reported to activate glycogen synthesis, modulate insulin signalling and regeneration of insulin action. They were also reported to suppress gluconeogenesis and also to suppress the activity of disaccharides [18]. Thus, since FAMT was found to possess all these phytochemical anti-diabetic agents, they are assessed to possess Anti-diabetic property.

3.4 Total Polyphenol Estimation

Polyphenols were reported to be anti-inflammatory and anti-oxidant property. They also have been reported to inhibit digestive enzymes. These properties made them induce a hypoglycaemic effect in the body when ingested [19]. It was observed that the total polyphenol level in FAMT extract was higher than almond milk and normal fermented almond milk (Table 4 and Fig. 3).

3.5 *In Vitro* Antidiabetic Analysis

3.5.1 DPPH scavenging activity

It was observed as in Fig. 4 that DPPH scavenging activity was significantly high in FAMT extract (59 ± 4%) than in Fermented almond milk (32 ± 2%). Siddhuraju et al. [20] has reported an antioxidant study on Indian Laburnum. It was reported that a higher scavenging activity would be an result of high level of phytocompounds like phenols and flavonoids. Thus, it can be concluded that the DPPH scavenging activity of FAMT was because of its presence of phenols and flavonoids. According to Brand et al. [14], the presence of bountiful amount of hydrogen donating phytocompounds were present in FAMT extract. Due to those hydrogen donating compounds (anti-oxidants), DPPH had accepted electrons and have changed the solution from violet to yellow (indication).

3.5.2 α -glucosidase inhibition assay

α -glucosidases are classes of enzymes that acts by breaking down starch and disaccharides into glucose. Thus, inhibition of these enzymes leads to lower rates of carbohydrate digestion resulting in less glucose availability for absorption [21].

It was observed as in Fig. 5 that α-glucosidase inhibition by FAMT extract (50 ± 4%) was significantly high when compared to Fermented almond milk (45 ± 2%). It was reported that polyphenolic phytocompound have the ability to bind with carbohydrate metabolising enzymes like α-glucosidase and inhibit them [22]. It is known that α-amylase is involved in hydrolysis of α-1,4-glycosidic linkages in starch to di and tri saccharides while α-glucosidase is involved in hydrolysis of those oligo-saccharides to monosaccharides. Since carbohydrates in the food material are hydrolysed by α-amylase and α-glucosidase, inhibition of these enzymes plays major role in reducing the glucose level in the body.

3.5.3 α -Amylase inhibitory activity

In humans, the digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers. Later on in the gut these are further hydrolysed by pancreatic α-amylases into maltose, maltotriose and small malto-oligosaccharides. The digestive enzyme (α-amylase) is responsible for hydrolysing dietary starch (maltose), which breaks down into glucose prior to absorption [21], [23]. Thus, Inhibition of α-amylase leads to reduction in post prandial hyperglycaemia in diabetic condition.

It was observed as in Fig. 6 that α-amylase inhibition by FAMT extract (52 ± 3%,) was significantly high when compared to Fermented almond milk (34 ± 2%). According to Hassan et al.,2017 [22] polyphenols were also reported to inhibit α -Amylase. Thus, inhibition of α-amylase would greatly reduce the glucose level in the body.

3.6 *In vivo* Animal Studies

It was reported that in diabetic animals, multiple mechanisms are thought to function synergistically to lower plasma glucose levels. Some plants have an oral antihyperglycemic property similar to sulfonylureas, which reduces the amount of blood glucose in normal animals, whereas other antihyperglycemics have no effect on normal plasma glucose levels and only show effects on diabetic animals like biguanides (metformin) [24].

It was observed as in Fig. 7 that Plasma glucose was significantly ($P<0.0001$) increased in positive control group upon the induction of STZ. This might be due to the destruction of pancreatic cells. It was observed that plasma glucose was significantly ($P<0.0001$) reduced in 5% FAMT treated groups when compared to positive control group (Group-II). It was also noted that 5% FAMT treated group showed better plasma glucose reduction than 5% fermented almond milk treated group ($P<0.001$). Since, FAMT was most likely to have a function like metformin, it did not show any effect on plasma glucose level in normal-glycaemic rats.

It was reported earlier that the decreased serum insulin level in STZ diabetic rats was due to the loss of pancreatic beta cells [25].

It was observed as in Fig. 8 that serum insulin was significantly ($P<0.0001$) lowered in positive control group upon the induction of STZ. It was noted that 5% FAMT treated group ($P<0.001$) showed significant serum insulin increase than 5% fermented almond milk treated group

(P<0.01) when compared to positive control group (Group-II). And the reason for increase in serum insulin could have been either by enhancing the insulin secretion of the remaining beta cells or by regenerating the pancreatic beta cells.

It was reported earlier that hyperglycaemia is accompanied by hyperlipidaemia in diabetes, according to several scientific reports. The key risk factor for coronary heart disease is elevated cholesterol levels. In the diabetic control group, triglycerides and cholesterol levels were elevated, which is related to a major improvement in lipid metabolism and structure. It was also reported that cholesterol cell metabolism disorders are responsible for high cholesterol changes in diabetes, and the authentic mechanism that caused these changes was determined [26].

A significant (P<0.0001) increase in serum Triglyceride was observed in positive control group upon the induction of STZ. It was noted that 5% FAMT treated group (P<0.001) showed significant serum TG reduction than 5% fermented almond milk treated group (P<0.01) when compared to positive control group (Fig. 9).

Table 1. Grouping of animals

Group	Description
Group I (G1)	Serve as negative control and will receive regular rat food and drinking water ad libitum
Group II (G2)	Serve as Streptozotocin induced diabetic model Only STZ induction on 29th day
Group III (G3)	pre-treatment with Metformin 350 mg/kg, p.o/ day for 28 days. STZ induction on 29th day
Group IV (G4)	pre-treatment with (5% of body weight) of Fermented Almond milk. STZ induction on 29th day
Group V (G5)	pre-treatment with (5% of body weight) of FAMT. STZ induction on 29th day

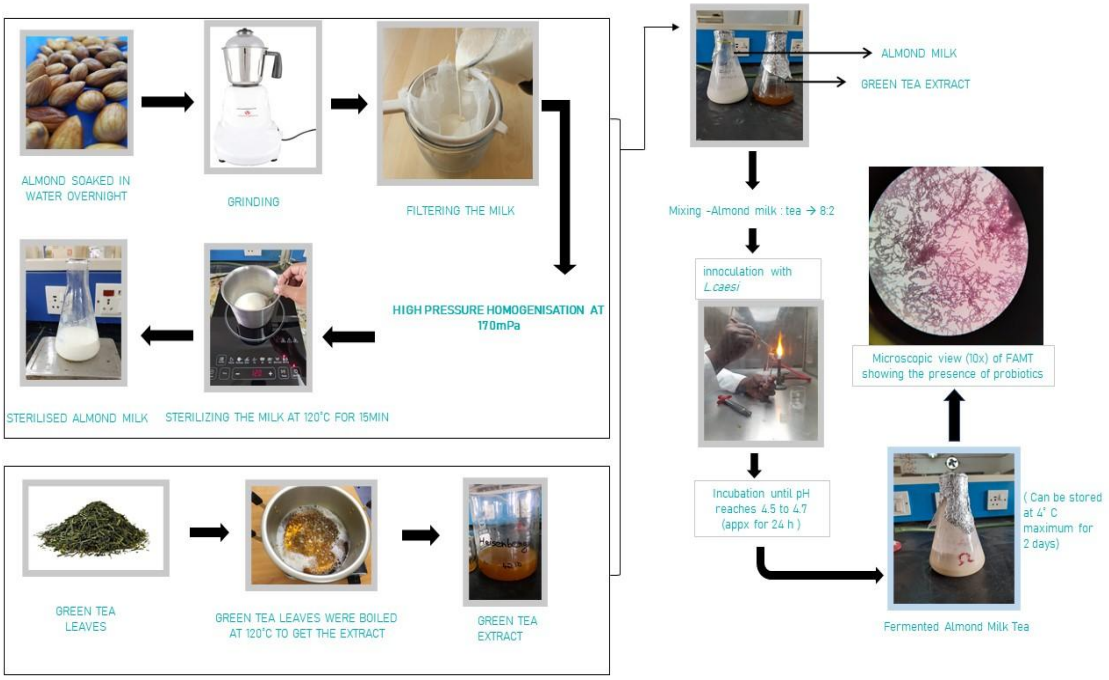

Fig. 1. Overall process of production of FAMT

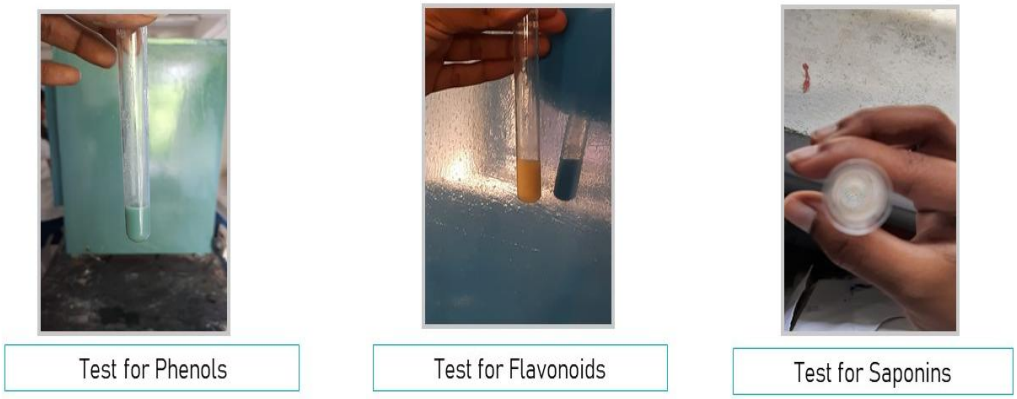

Fig. 2. Pictorial evidence confirming the presence of phytochemical in FAMT

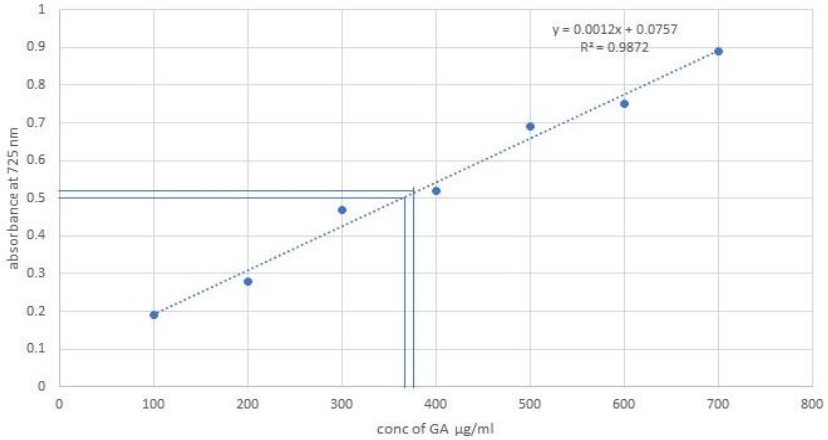

Fig. 3. Estimation of total polyphenol in FAMT using gallic acid standard graph
Continuous blue line indicates the duplicates absorbance value of the unknown FAMT extract sample

Table 2. Sensory analysis of FAMT

Parameter	Fermented almond milk	FAMT (8:2)	FAMT (5:5)	FAMT (4:6)
Flavor	6.5±2.45	7.4±2.37	6.88±1.31	6.05±0.33
Taste	6.6±2.37	7.45±1.41	6.88±1.31	5.62±2.46
Aroma	6.45±1.41	7.3±3.43	7.97±2.25	8.21±1.27
Overall acceptability	6.55±1.41	7.45±1.01	6.77±1.41	5.93±2.41

Table 3. Preliminary qualitative phytochemical analysis of FAMT

S. No	Phyto chemical analysis	Observation	Result
1	Test for Phenols	Appearance of blue colour solution	+
2	Test for Flavonoids	Yellow solutions	+
3	Test for Saponins	Appearance of foam	+

+ indicates the presence of the bio-actives

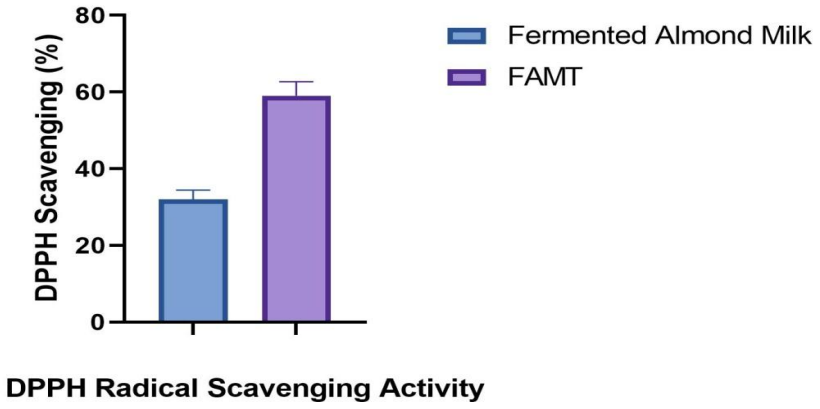

Fig. 4. DPPH scavenging activity
Values were expressed in mean ± SEM (n=3)

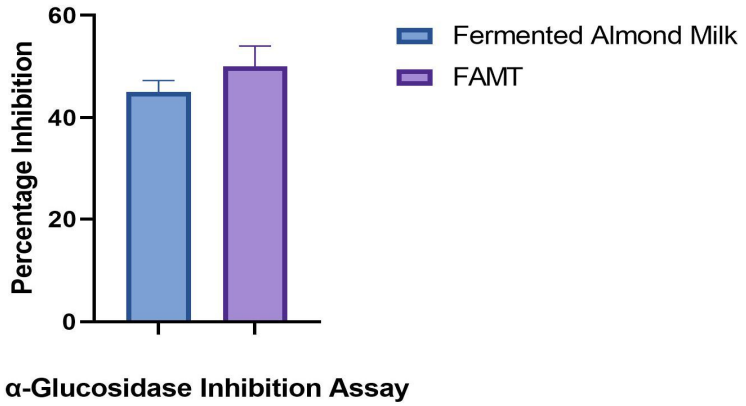

Fig. 5. (α)-Glucosidase inhibitory activity
Values were expressed in mean ± SEM (n=3)

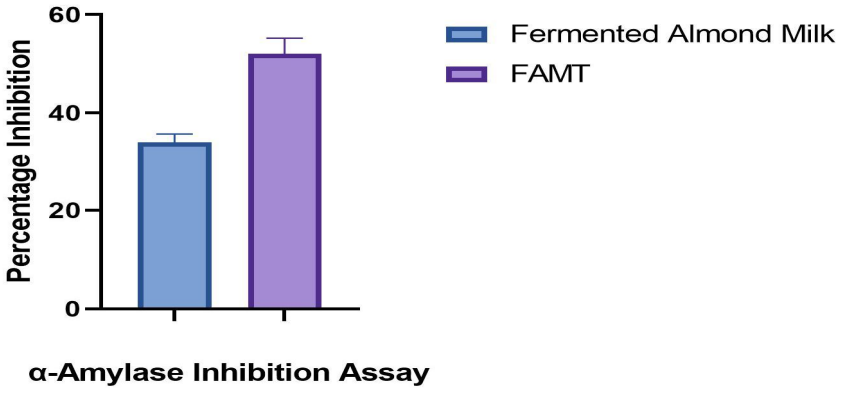

Fig. 6. (α)-Amylase inhibitory activity
Values were expressed in mean ± SEM (n=3)

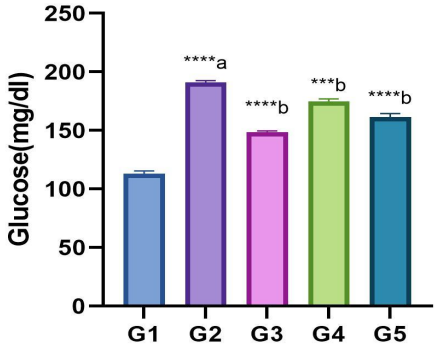

Fig. 7. Plasma glucose level of the experimental animals

*Values were expressed in mean ± SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant*

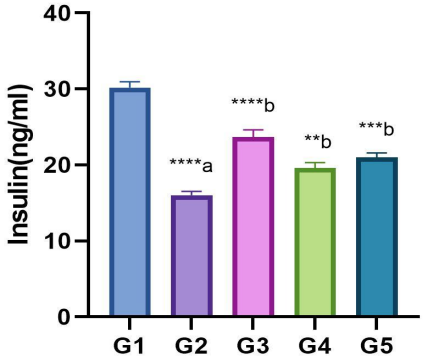

Fig. 8. Serum insulin level of the experimental animals

*Values were expressed in mean ± SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant*

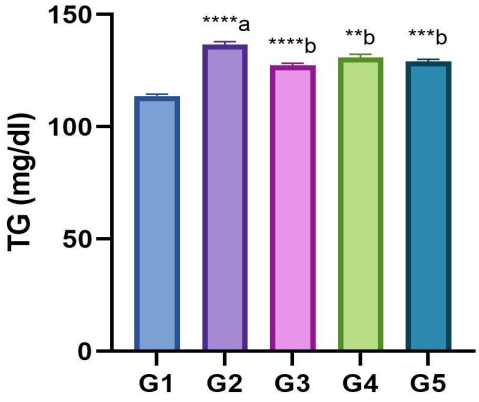

Fig. 9. Serum TG level of the experimental animals

*Values were expressed in mean ± SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant*

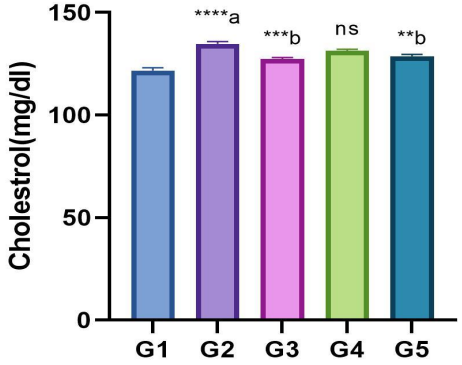

Fig. 10. Serum cholesterol level of the experimental animals

*Values were expressed in mean ± SEM (n=6), *P<0.05, **P<0.01, ***0.001<P<0.0001, ****P<0.0001; a significant compared with control group, b significant compared with calculi induced group, ns not significant*

Table 4. Estimation of total polyphenol in different almond products

Duplicates	Almond milk (µg/ml)	Fermented almond milk (µg/ml)	FAMT (µg/ml)
D1	210	230	370
D2	218	235	376
Mean ± SEM	214 ± 4.0	232.5 ± 2.50	373 ± 3.0

Values were expressed in mean ± SEM (n=3)

A significant (P<0.0001) increase in serum cholesterol was observed in positive control group upon the induction of STZ. It was noted that 5% FAMT treated group (P<0.01) showed significant serum cholesterol when compared to positive control group (Fig. 10).

4. CONCLUSION

Based on the above study, it can be concluded that FAMT can be considered as a potent diabetes prevention drink. The main mechanism behind it could be due to the role of bioactive phytocompounds- phenols, flavonoids and saponins, especially the polyphenols in forming a protective layer over the pancreas, leading to the prevention of pancreatic cell damage from external free radicals or any other causative agents. It has a potent free radical scavenging activity than normal fermented almond milk. It is found that FAMT extract was able to inhibit glucose metabolising enzymes like amylase and glucosidase. This could greatly help them attain the property of reducing glucose level in the blood. It was also evident through animal studies that it produces better reduction of plasma glucose, serum TG and serum cholesterol and increases the serum insulin level than that is resulted by normal fermented almond milk. Further clinical trials have to be performed to extrapolate its efficiency of preventing Diabetes mellitus in Humans.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It was obtained from volunteer for sec. 3.2.

ETHICAL APROVAL

Animals approval for Protocol-1/Phase-2/2019 was sanctioned in 11th IAEC meeting held on 23.12.2019 at Sri Venkateswara College of Engineering (Reg. No: 1398/PO/Re/S/10/ CPCSEA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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