



Title: Assessment of Honey for its composition of probiotic lactic acid bacteria and their effects on consumption by Diabetic patient using Wistar rat

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Abstract

The relevance of probiotic, especially lactic acid bacteria cannot be over emphasized. In this present study three honey sources were serially diluted and cultured on De Man, Rogosa and Sharpe (MRS) agar among which only one of this sources grew on MRS agar. The pure Lactobacilli isolate were subjected to gram staining, biochemical tests, physiological test, molecular analysis using Polymerase Chain Reaction (PCR) techniques and Deoxyribonucleotide (DNA) sequencing. The isolates obtained comprising of many yeast (*Saccharomyces cerevisiae*) and a single probiotic lactic acid bacteria known as *Enterococcus fecalis*. The strain obtained is similar to an already identified probiotic lactic acid bacteria from the gene bank known as OR271976 *Enterococcus fecalis* with an evolutionary similarity of 100%. The isolate was subjected to probiotic selection and was found fit for consumption. Honey found to be harbouring *Enterococcus fecalis* as a probiotic were further then used for the treatment of Type-2 diabetic rats that has been induced with Streptozotocin injection at a dose of 65mg/kg body weight at a single dose intraperitoneally, but LD₅₀ (Lethal dose) of 79.5mg/kg was observed at a single dose. Honey was not found to be effective in reducing the fasting blood glucose (FBG) level, but rather increased it with a significant increase difference of 58.9mg/dL when compared at 0 and 4 weeks (from 241.4mg/dL to 300.3mg/dL) of treatment. Based on the outcome of this study, diabetic patient are advised not to consume honey. However, based on this study, honey can also be beneficial in reducing weight, basal mass index, lee's index and total blood cholesterol level especially in obsessed and hypercholesterolemic patient. However, no side effect was discovered within the four weeks of administration (treatment) apart from his alarming side effects on the fasting blood glucose level.

Introduction

In the bodies of human and animal, lactic acid bacteria (LAB) are part of normal microbiota of gastrointestinal tract and genitourinary tracts. They are gram positive bacteria, non-spore former, carbohydrate fermenter, aerotolerant anaerobes, catalase and oxidase negative. They have been given the Qualified Presumption of Safety (QPS) status.

They belong mostly to family of Lactobacillaceae. Majority of probiotics are lactic acid bacteria. The sources of Lactic acid bacteria (LAB) are mostly fermented and dairy food. The isolation of Lactic Acid Bacteria involve culturing of the potential source in a selective medium such as de Man, Rogosa, and Sharpe medium commonly known as MRS agar in an anaerobic condition (Razmposh *et al.*, 2016).

Statement of Problems

The relevance of honey to human cannot be overemphasized. Honey is presently being used for wound healing. Although, the rationale behind its use for wound healing could be due to the fact that it harbors some probiotic lactic acid bacteria which can be used as an alternative to antibiotic treatment. However, their effect on metabolic disease such as diabetes when consumed orally has not been scientifically claimed. Hence there is need to isolate and characterize probiotic lactic acid bacteria that could be present in honey and the effect of honey consumption by type-2 diabetic patient by assessing some clinical parameters.

Material and Methods

Sample Source and Inclusion and Exclusion Criteria

Honey of three different producers which are original and not expired was selected for the research study.

Sample Collection

The Honey were collected into a universal sterile container observing universal sterile technique using sterile syringes, methylated spirit and then analysis was commenced immediately.

Sample Processing, Phenotypic Diagnosis and characterization of Lactic acid bacteria

Sample processing

One millilitre (1ml) of the honey from three different producers was collected from the containers using sterile needle and syringe under universal aseptic technique. A serial dilution of 10^{-3} of the three different brands of honey using sterile water solution (Microxpress[®], a division of Tulip Diagnostics Ltd.) and incubated at 25°C for 30 minutes. Streak plating method was used as described by Sun-young and his colleagues with some slight modification (Sun-young *et al.*, 2019). Then 100µL from each dilution of the samples was inoculated and cultured into de Man, Rogosa, and Sharpe (MRS; TITAN Biotech Ltd., Rajasthan, India) agar using micropipette and incubated at 37°C for 24 hours to 72 hours anaerobically to obtain different colonies and then purified by streak plate techniques on MRS agar for subculture and incubated at 37°C for 24hours to 48hours anaerobically again to obtain pure culture. The pure colony from each sample was further inoculated into MRS broth containing 15% glycerol and stored at – 20°C to preserve the isolates for DNA extraction, sequencing and in-vivo evaluation (Nabi *et al.*, 2020).

Phenotypic Diagnosis of Presumptive LAB

Pure culture of Presumptive LAB plate immediately after isolation was assessed for colony morphology and was further characterized microscopically via gram stain reaction and biochemically via oxidase, catalase and carbohydrate fermentation test using Mohan and Murugalatha method. Microscopically, they are gram positive

bacteria with rod-shaped or cocci-shaped. Biochemically, presumptive Lactic Acid Bacteria colonies are catalase negative and oxidase negative or positive; and carbohydrate fermentation test is positive (Forhad *et al.*, 2015). For additional confirmation of LAB, temperature and sodium chloride tolerance test were done. A loop full of suspected LAB was inoculated into 10mls of MRS broth and examined for their ability to grow at different temperatures (15, 37, 45°C) for three (3) days and saline concentration (2, 3, 4, and 6.5% (w/v) NaCl) for 24 to 48hours and then incubated at 37°C anaerobically. Both procedures was described by Estifanos (Estifanos, 2014), observing their turbidity rate. Lactic acid bacteria that show tolerance to the subjected temperatures and different saline concentration was confirmed as LAB and then stored in MRS broth containing 15% of glycerol at -10°C prior further analysis (Davodabadi *et al.*, 2015).

Molecular Identification of the Isolated Presumptive Probiotic LAB

The DNA extraction was done using an extraction kit on ZR fungal/bacterial DNA mini prep (Inqaba in South Africa).The DNA extracted was quantified using a spectrophotometer called Nanodrop 1000 spectrophotometer. The amplification of the DNA was done using the polymerase chain reaction (PCR) machines operating at 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers, followed by checking for purification using agarose gel electrophoresis technique (Pei *et al.*, 2012). The Sequencing of LAB was done using a machine known as a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The PCR template used is per 100 base pair (Anna *et al.*, 2017).

Characterization of Functional and Safety Properties of Confirmed Probiotic LAB Isolates

Acid Tolerance Assay

Each confirmed LAB isolates obtained from overnight culture in MRS agar was cultured overnight in MRS broth at 37°C and settled by centrifugation for 10min at 5000rpm. The supernatant was discarded while it precipitate containing suspension of grown confirmed LAB isolates was left in the test tube. One normality of hydrochloric acid was used to modify the precipitate to obtain a final pH of 2.5 as measured by pH meter and then incubated anaerobically at 37°C for 2 hours and assessed for growth by assessing their turbidity in each tube. To further evaluate the resistant of confirmed LAB to acid by being turbid, the methodology used by Rajesh and his colleagues was applied with slight modification where hundred microliters (100µL) of the bacteria culture from those test tubes that are turbid was inoculated in MRS agar in duplicates and examined after 24 to 48 hours of incubation anaerobically at 37°C. Those inoculated plate which showed growth of more 10^7 CFU/ml (number of colonies of bacteria in each plate was between 5 and 20) were considered as resistant to acid (Rajesh *et al.*, 2016).

Bile Tolerance Assay

Bile tolerance was evaluated through growth studies by the method of Succi and his colleagues with some modifications in 2005 where hundred microliters (100µL) of bacterial culture of each isolates of confirmed LAB isolates was transferred to 9mL of modified MRS broth containing 0.15 and 0.3% bile salt concentration and then incubated at 37°C anaerobically and viable growth on MRS broth was assessed for turbidity after 0, 4, 24, and 48hours and their ability to reduce the pH (pH meter, Eutech instrument, Malaysia) of the modified MRS broth

solution at 0, 4, 24 and 48 hours of incubation. Those test tubes showing turbidity and reduced pH of the modified MRS broth were considered to be resistant to bile salt (Succi *et al.*, 2005).

Safety Assay

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was carried out with Muller Hinton agar diffusion by Kirby Buer method (phenotypic methods) using commercially available antibiotic discs (MAST, Berkshire, UK) in Nigeria as described by Vijayakumar and his colleagues in 2015. The antibiotic disc includes amoxicillin (AML, 20µg), norfloxacin (NB, 10µg), streptomycin (S, 30µg), levofloxacin (LEV, 20µg), chloramphenicol (CHL, 30µg), ciprofloxacin (CIP, 10µg), rifampicin (RIF, 20µg), erythromycin (ERY, 30µg), ampiclox (APX, 20µg) and gentamicin (CN, 10 µg). After streaking, antibiotic discs of gram positive disc were placed on the inoculated MHA agar surface using sterile forceps ensuring that each disc is gently pressed down to ensure complete contact with the agar and the plate was left over for 20 min at 4°C for diffusion of antibiotics and then incubated at 37°C for 24 to 48 hours under anaerobic condition, after which the inhibition zone diameters were measured with vernier caliper and results were (Vijayakumar *et al.*, 2015). *Lactobacillus bulgaricus* were used for positive quality control.

Hemolytic Test

To assess their safety via hemolytic testing, Yasmin and his colleague's method in 2020 was used. Fresh overnight confirmed LAB isolates were streaked on Human blood agar and incubated at 37°C for 24 to 48 hours in an anaerobic condition. Hemolytic activity of the confirmed LAB isolates was examined for alpha, beta and gamma hemolysis. Probiotic LAB undergoes gamma-hemolysis (Yasmin *et al.*, 2020).

In-vivo Evaluation of Honey for its Anti-diabetic Properties

Ethical consideration and study design

The rules guiding the use of animal for experimental study were strictly adhered to after ethical consent was granted. This research utilized randomized subject control experimental design and observational study. Eighteen (18) adult male wistar rats (*Rattus norvegicus*) were randomly divided into two (2) main groups. The two main groups are normal group and diabetes induced group. The diabetes-induced group was further subdivided into two (2) subgroups namely diabetic group on treatment and those not on treatment. Prior experimental design, a pilot study was done on the standardization of streptozocin injection required to induce type-2 diabetes on the adult wistar rat and the LD50 of streptozocin injection.

Management of Experimental Animals

The rat was maintained under standard laboratory conditions in the animal house. The cages used for keeping the rat were measuring 38×23×15cm with not more than seven rats per cage. They were fed with pelletized growers feed. Prior treatment, the wistar rats were fed with goya oil as a source of high fat diet to induce obesity via gastric gavage. Obesity in wistar rat was assessed using two anthropometrical parameters such as Body mass index (BMI) and Lee index. The formula used for calculating the basal mass index and Lee index are given below.

$$\text{Body Mass Index} = \frac{\text{Body weight (g)}}{\text{Length}^2(\text{cm}^2)}$$

$$\text{Lee's Index} = \frac{\text{Cube root of body weight (g)} \times 1000}{\text{Nose - to - anus length (cm)}}$$

Obesity is estimated in rat using BMI and lee's index. The normal BMI for rat is between 0.45g/cm² and 0.68g/cm² while the normal Lee's index for rat is less than 300g/cm (Novelli *et al.*, 2006).

Parameters evaluation and Treatment of Animals

Prior administration of Streptozotocin intraperitoneally at lower left or right quadrant of the abdomen, the anthropometric parameter (basal mass index and Lee's index), biochemical parameters (blood glucose, total cholesterol) and hematological parameter (packed cell volume) was estimated before and seven (7) days after Streptozotocin administration. The standardized dose of streptozotocin injection used to induce diabetes is 65mg/Kg body weight and the lethal dose 50% was 79.5% obtained via a pilot study conducted prior experimental design. The male adult wistar rats with blood glucose of greater or equal to 180mg/dL and total serum cholesterol level of greater than or equal to 100mg/dL; and change in body weight via assessment of their BMI/ Lee index (5% decrease of initial weight) were confirmed for the presence of obesity and metabolic syndrome with type-2-diabetes mellitus. Blood samples were collected from the tail vein at 0, 4, 6 and 8 weeks for estimation of biochemical parameters. Treatment was carried out for four (4) weeks (Rajesh *et al.*, 2016). The treatment were divided into two groups namely:

Group A: Normal Study. The rats in this group was fed with HFD, normal rat feed and water orally daily via gastric gavage but were not induced with Type-2 diabetes

Group B: Diabetes-Induced Study

Group B subgroup I: Control. The rats in this group are diabetic-induced and were given commercial feeds and treated with 0.9% physiological saline (3mL/Kg/BW) orally daily via gastric gavage. They serve as positive control (Rajesh *et al.*, 2016).

Group B subgroup II: Honey (3mL/Kg/BW). The rats in this group are diabetic-induced and were given commercial feeds and treated with honey (3mL/Kg/BW) orally daily via gastric gavage. This was done to also assess the glycemic index of honey. The treatment was preserved in a refrigerator after each use daily.

Each parameter were estimated using appropriate device and instruments such as glucometer (Accu-check), urinalysis combi-9 strips and automated four in one machine (Accu-answer) that assess total cholesterol level, fasting blood sugar, uric acid and packed cell volume with separate strips.

Statistical Analysis

All the measurements were performed in triplicates and the results (data) were expressed as mean ± standard deviation (SD).

Results and discussion

Table 4.1 showed that out of the entire three (3) different honey source used, only one (vickag's honey) of them grown on MRS agar after incubation anaerobically at 37°C for 24 to 48hours of incubation having a colony size of 0.5mm and designated as J₁.

Table 4.1: Isolation of presumptive Lactic acid producers from samples collected

Market (s)	Fermented food (s)	No of colonies	No. of isolates
Market square	Vickag's honey	1	J ₁
	Bema honey, Omphalos honey	0	
Total	3	1	1

The isolate obtained was subjected to gram stain, catalase test, oxidase test and carbohydrate fermentation test. This is illustrated in plate 1 and figure 1. The isolate was confirmed gram positive bacteria with cocci shape, catalase-negative, oxidase-negative and sugar fermenters (lactose and dextrose). The isolate was further subjected to physiological characterization using temperature and saline (sodium chloride) tolerance test as showed in table 4.2. The bacterium did not grow at a temperature of 10°C, but rather grew optimally at a temperature of 37°C and 45°C. The isolate grew optimally at a saline concentration of 2%, 4% and 6.5% that's has been incorporated into MRS agar. After DNA sequencing and agarose gel electrophoresis was done, the isolate obtained was confirmed probiotics known as *Enterococcus faecalis*.

Table 4.2: Microscopic, biochemical and physiological characterization of Lactic acid Bacterium isolated

Tests/Isolates	<i>Enterococcus faecalis</i>
Morphology	Cocci
Biochemical testing	
Catalase	Negative
Oxidase	Negative
Carbohydrate fermentation	
Glucose	Positive
Lactose	Positive
Physiological growth at different temperature	
10°C	Negative
37°C	Positive
45°C	Positive
Growth at different NaCl concentration	

2%	Positive
4%	Positive
6.5%	Positive

The sequence homologies detected through phylogenetic analysis showed *Enterococcus faecalis* has 100% similarity with an already previously identified OR271976 *Enterococcus faecalis* from the gene bank. **Table 4.3, 4.4, 4.5, and 4.6** showed the tolerance of the confirmed LAB to acid and bile salt. Table 4.3 shows the susceptibility of *Enterococcus faecalis* cells after being inoculated in MRS broth modified with hydrochloric acid of pH 2.5 and bile salt at a concentration of 0.15 and 0.3%. The two modified media will be incubated at 37°C anaerobically and examined for turbidity at 0hr and 24hour. The isolate was showed to be acid and bile tolerant.

Table 4.3: Probiotic characteristics (acid and bile tolerance test) of *Enterococcus faecalis*

Inhibitory condition/ LAB isolates	<i>Enterococcus faecalis</i>
Acid tolerance test	+
pH 2.5 at 0hour	+
pH 2.5 at 24hours	+
Bile tolerance test	+
0.15% bile at 0hour	+
0.15% bile at 6hours	+
0.15% bile at 24ouhrs	+
0.15% bile at 48hours	+
0.3% bile at 0hour	+
0.3% bile at 6hours	+
0.3% bile at 12hours	+
0.3% bile at 24hours	+
0.3% bile at 48hours	+

Table 4.4 showed that *Enterococcus faecalis* isolated from Vickags honey had bacterial count (no bacterial growth) after its inoculum from the modified MRS broth with a pH of 2.5 after 24hours was inoculated into MRS agar and incubated anaerobically at 37°C for 24 to 48hours.

Table 4.4: Acid tolerance test of *Enterococcus faecalis* on MRS agar after incubation in MRS broth modified with acid

Isolates	Log ₁₀ CFU/mL	
	Bacterial count at 0hour (×10 ⁷)	Bacterial count at 24hours (×10 ⁷)
<i>Enterococcus faecalis</i>	5.50 ±0.10	5.20 ±0.23

A mean ± standard deviation of 0.00 ± 0.00 signifies that those isolates had no growth (no bacterial count).

Table 4.5 and 4.6 showed the results of the analysis of pH values of the inoculated MRS supplemented with 0.15 and 0.3% (w/v) bile salt interpreted via the use of the mean or standard deviation. In MRS without bile, the inoculum (*Enterococcus faecalis* cell) grew faster than MRS supplemented with 0.15% and 0.3% bile at every sampling time (0, 4, 6, 24 and 48 hours) with growth appearing after 24hours of inoculation. *Enterococcus faecalis* showed attribute in lowering the pH of the MRS agar that has been supplemented with 0.15% and 0.3% bile salt medium when compared with at 0 hour and 48hours of inoculation.

Table 4.5: Tolerance to 0.15% bile salt of lactic acid bacteria isolated

Culture media/ Sampling time (hours)	MRS agar + 0.15% Bile salt			
	0	6	24	48
<i>Enterococcus faecalis</i>	5.70±0.00	5.68±0.01	5.64±0.20	5.60±0.00

Table 4.9 showed the antibiotic susceptibility testing (which was carried out with Muller Hinton agar diffusion with Kirby Buer method) of *Enterococcus faecalis* that was assessed through determination with minimum inhibitory concentration (MIC) of ten common (10) antibiotics. The ten antibiotics include amoxicillin (AML, 20µg), norfloxacin (NB, 10µg), streptomycin (S, 30µg), levofloxacin (LEV, 20µg), chloramphenicol (CHL, 30 µg), ciprofloxacin (CIP, 10µg), rifampicin (RIF, 20µg) erythromycin (ERY, 30µg), ampiclox (APX, 20µg) and gentamicin (CN, 10 µg). *Enterococcus faecalis* cells were all susceptible to all the ten antibiotics.

Table 4.7: Antimicrobial susceptibility test of the confirmed *Enterococcus faecalis*

Antibiotics	Disc potency (µg)	Isolates <i>Enterococcus faecalis</i>
Amoxicillin	20	S
Norfloxacin	10	S
Streptomycin	30	S
Levofloxacin	20	S
Chloramphenicol	30	S
Ciprofloxacin	10	S
Rifampicin	20	S
Erythromycin	30	S
Ampiclox	20	S
Gentamycin	10	S

Where inhibition zone diameter

$\leq 8\text{mm}$ = Resistant (R)

8–10mm= Moderate susceptibility (M)

$\geq 10\text{mm}$ = susceptibility (S)

Finally hemolytic assay test confirmed that this strain of *Enterococcus faecalis* isolated from vickag's honey was gamma hemolytic. These means they are safe for consumption by human.

Table 4.8 shows the effect of Honey on some anthropometric (weight, body mass index and Lee's index), biochemical (total cholesterol, fasting blood glucose), hematological (Packed cell volume) and microbiological parameter (glucosuria and proteinuria) at four (4) weeks. However, the standardized value and LD₅₀ value obtained for this present study is 65mg/kg body weight and 80mg/kg body weight respectively.

The FBG of T2D rat fed with honey had significant increase in their FBG (from 241 to 300mg/dL) compared to normal (non-diabetic) (82.8 to 85.4mg/dL) rat and T2D induced rat (220 to 300.3mg/dL). The weight and Total cholesterol level of T2D rat fed with honey decreased significantly. The PCV of those T2D rat fed with honey increased when compared to T2D induced rat (60 to 63.5%).



Plate 1.: Showing the growth of *Enterococcus faecalis* on MRS agar

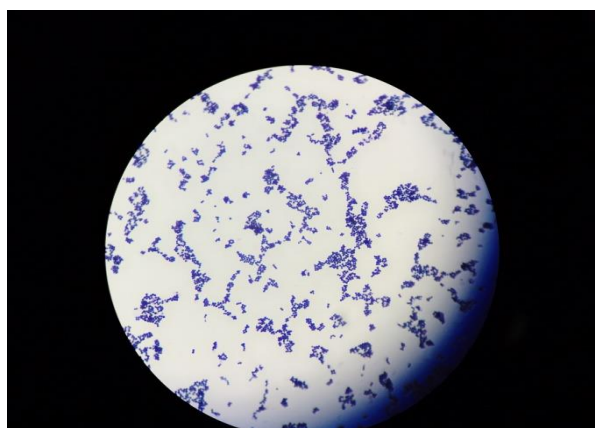


Figure 1: Showing *Enterococcus faecalis* on gram stain

Discussion

Only 1 (33.3%) of the honey out of three different source of honey (100%) used had growth on MRS agar. The reasons why the entire different honey source used in this study did not grow on MRS agar, could be due to some physical factors that can affect probiotic survival such as temperature, temperature of fermentation and pH according to Karin and Barbel (2000).

The bacterium isolated fit the classification of lactic acid bacteria (LAB) as gram positive, catalase-negative, oxidase-negative and carbohydrate fermentation-positive. This finding is in accordance with Akalu *et al.*(2015) who isolated *Lactobacillus* species from Ergo (pickled vegetable). The LAB isolated in this study grew well at 37°C and 45°C after incubation period of 24 to 48 hours which is the optimum temperature range for their growth. However, there was no growth of these isolates at 10°C. This is similar to the study of Ayodeji *et al.* (2020). The isolate obtained in this study grew well at 4.0 % and 6.5% salt concentration. Amplified DNA of the Lactobacilli bacteria isolates were sequenced and the results were compared with the ones in Genebank database using local alignment search tool (BLAST) for complete identification which came out to be *Enterococcus faecalis*, This is support with the work of Abdulmumini *et al.*(2021) which isolated Lactic bacteria from cabbage and Soumitra, (2020) from traditional fermented rice. The bacterium isolated and subjected to a pH of 2.5 at 0 and 24hours showed tolerance to pH of 2.5 for 0 and 24hours. Chan *et al.*(2011) reported that acids such as the hydrochloric acid (HCl) found also in human stomach, disrupt the biomolecules of cells, such as fatty acids , proteins and DNA. Our findings are consistent with previous studies where *Weissella confusa* demonstrated its ability to survive in high acidic environments. The bacterium isolated in this study exhibited high resistance to bile concentrations of 0.15% and 0.3% at 0, 6, 24 and 48hours with reduction in pH of MRS broth. Based on Chateau *et al.* (2011) classification of lactobacilli based on their delay in their growth in the presence of bile salt, the bacterium isolated in this study are sensitive to bile salt.

The LAB isolated was found to be susceptible to all the antibiotics. The outcome of the antibiotic susceptibility testing in this study is not similar to the work of Casado *et al.* (2014). The LAB isolated in this study was non-hemolytic and as such they were not selected for other tests since their safety were confirmed. This is not in accordance with the work done by Olufemi *et al.* (2018) where most LAB strains they isolated were non-hemolytic. Table 4.10 shows Comparism of some Anthropometric, biochemical, and hematological parameter of normal, control and treatment group. However, the fasting blood glucose of Type-2 diabetes rats treated with Honey (from 241.4mg/dL as at 0 week from 300.3mg/dL as at fourth week) when compared to the fasting blood glucose (FBG) of control group was high. In this present, *Enterococcus faecalis* was isolated from honey. Honey has been found to harbor *Enterococcus faecalis* in previous researches. This probiotic in honey in previous study has been found to regulate in Honey bee developmental genes (Yating *et al.*, 2021). The result of this present study obtained from the effect of honey consumption on T2D rat is in concordance with the some previous research such as those obtained by Marzieh and his colleagues (2021), they stated that high intake of honey might increase glucose levels and worsen other metabolic parameters in patients with Type-2 diabetic mellitus (T2DM).

Table 4.10: Comparing some Anthropometric, biochemical, and hematological parameter of normal, control and treatment group

Parameter (s)	Experimental group					
	Normal group		Control group		treatment group	
	0week	4weeks	0week	4weeks	0week	4weeks
APM						
Wt(g)	209.8±35.3	229.6±13.77	227.8±1.70	220.8±13.83	180±9.2	169.8±1.83
BMI						
(g/cm ³)	0.54±0.04	0.59±0.04	0.53±0.03	0.5±0.06	0.53±0.03	0.5±0.06
LI (g/cm)	307.3±5.5	351.6±6.51	298.6±7.0	288.6±12.4	270.6±7.0	261.6±12.4
BCM						
FBG (mg/dL)	82.8.3±8.2	85.4±5.1	220±96.6	262.3.±27.9	241.4±96.6	300.3±12.7
TC (mg/dL)	95±7.0	97.2±2.1	123.8±14.5	220.8±13.83	102.3±4.4	93.7±2.5
HEM.						
PCV (%)	62.8±4.1	63.7±23.08	60±2.6	53.5±5.05	61±2.6	63.5±2.05

Key:

APM= Anthropometric parameter; Wt = Weight ;BMI= Body mass index ;LI= Lees index

BCM= Biochemical parameter ;FBG= Fasting blood glucose ;TC= Total cholesterol

HEM= Hematological parameter ; PCV= Packed cell volume; %= percentage

mg/dL=milligram per decilitre ; g/cm= gram per centimeter; g/cm³

Conclusion and recommendation

In conclusion, honey harbor *Enterococcus fecalis*, a probiotic lactic acid bacterium, however it was found to increase the glycemic index of type-2 diabetic wistar rat. Based on this present study, it is recommended that diabetic patient avoid the consumption of honey. Honey should be preserved in the refrigerator to ensure survival of the probiotic they harbor which might aid in wound healing and weight loss among obsessed patient. Furthermore, consumption of honey by non-diabetic patient have been found based on this study to decrease weight, basal mass index, lee's index and total blood cholesterol level. However, it has been found increase the packed cell volume in diabetic patient.

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