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Original article

Evaluation of the effect of Synbiotic in High-fat diet and streptozotocin induced diabetes in Wistar rats

Wadulkar R. D[1]. Bhusnure O.G.[2]

1. Dayanand College of Pharmacy Latur. (Maharashtra), India.
2. Channabasweshwar Pharmacy College, Latur. (Maharashtra), India.

Address for Correspondence:
Mr. Wadulkar R.D.
Dayanand College of Pharmacy, Latur
Email: rwadulkar29@gmail.com
Mob: 9421604150

Abstract:

Background: Dysbiosis is the causative factor for chronic diseases including diabetes, obesity, cancer, and Inflammatory Bowel disease. Homeostasis of gut flora with prebiotics and probiotics could reduce the risk factors of chronic disease.

Aim: To study the effect of Synbiotic (combination of prebiotics and probiotics) in a High-fat diet and STZ-induced diabetes in Rats.

Methods and Material: In the present study we designed the formulation containing sauerkraut juice as a good source of probiotics and prebiotics (*Allium Sativum and Cucurbita maxima)*. The designed polyherbal formulation (PHF) in lower and higher concentrations was evaluated for antidiabetic and antiobesity activity in a High-fat diet and Streptozotocin induced diabetes in Wistar rats.

Results: Polyherbal formulation in lower concentration (PHF-LC) and Polyherbal formulation in high concentration (PHF-HC) reduced blood glucose significantly ($p<0.01$) & ($p<0.001$) respectively when compared to the Diabetic (HFD-STZ) control group and positive control group. Reference standard metformin most effectively ($p<0.001$) reduced the blood glucose level when compared to the Diabetic (HFD-STZ) control group. It also shows a significant reduction in the lipid profile and body weight of animals. The histopathological study clearly shows that polyherbal formulation in higher concentrations restores the normal architecture of the liver and pancreas

Conclusions:

It was concluded that polyherbal combination in higher concentrations shows significant antiobesity and antidiabetic effects in High fat and STZ-induced diabetic rats. The polyherbal formulation may prevent dysbiosis-induced diabetes and obesity in Wistar rats. But further preclinical and clinical evaluation is required for the clinical use of such

formulation. Adding synbiotics to the diet could be a good approach to the prevention and treatment of chronic disease

Keywords: Synbiotics, Probiotics, High Fat diet, Streptozotocin, obesity.

Introduction

Chronic diseases are the leading causes of death and disability worldwide. Prevalence and occurrence of chronic diseases increasing worldwide, and pervading all socioeconomic classes.

More than 100,000 billion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the microbiota, has even been considered to be an important organ carrying approximately 150 times more genetic factor than are found in the entire human genome. (O'Hara & Shanahan, 2006) Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity (Ley et al., 2006).

Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota (Ley et al., 2006).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease via several mechanisms (Sekirov et al., 2010).

The human microbiota alters the host physiology to a great extent. Trillions of microbes colonize the human body, including bacteria, viruses, archaea, and eukaryotic microbes. The body contains at least a thousand different species of known bacteria and carries one fifty times more microbial genes than are found in the entire human genome (Ursell et al., 2014).

The prevalence of obesity has increased worldwide in the past 50 years, reaching pandemic levels. Obesity is the risk factor for different chronic such as type 2 diabetes mellitus, fatty liver disease, High blood pressure, myocardial infarction, stroke, dementia, osteoarthritis, obstructive sleep apnoea, and several cancers, thereby contributing to a decline in both quality of life and life expectancy. Obesity is also associated with unemployment, social disadvantages and reduced socio-economic productivity, thus increasingly creating an economic burden (Blüher, 2019). In terms of etiology, obesity is a multifactorial phenomenon, influenced by various genetic and environmental factors. Among the proposed environmental exposures, gut microbiome status has been extensively focused during recent decades (Khan et al., 2016).

The bacterial composition and diversity of the gut are altered in obese individuals, in comparison with lean individuals (Le Chatelier et al., 2013). Changes in microbial diversity and composition are increasingly associated with several disease states including obesity and behavioral disorders. Obesity-associated microbiota alters host energy harvesting, insulin resistance, inflammation, and fat deposition. Moreover, the intestinal microbiota can regulate digestion, adiposity, homoeostasis, and energy balance. Therefore, the gut microbiota is becoming a target for new anti-obesity therapies. (Dinan & Cryan, 2017)

Probiotics can be used both to prevent the onset of dysbiosis when the patient is exposed to predisposing conditions (prolonged antibiotic therapies, intense physical or mental stress, chronic debilitating diseases, etc.) and as therapeutic agents to rebalance an ongoing condition of dysbiosis (Gagliardi et al., 2018). Probiotics are among the new dietary approaches to keep the gut microbiota in a healthy state and modulate the gut bacterial imbalance. Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill et al., 2014). Several mechanisms have been proposed in relation to anti-obesity effect of probiotics, including improvement of gut barrier function, followed by reduced gut permeability, metabolic endotoxemia, and thus systemic inflammation, deconjugation of bile acids, which interfere with lipid absorption, and increasing the level of short chain fatty acids (SCFAs), results in stimulation the synthesis of some intestinal peptides involved in energy homeostasis and appetite regulation (Mills et al., 2018).

The growth and promotion of beneficial community of microbes by using functional foods such as prebiotics has been encouraged in recent years. (Parracho et al., 2007) Prebiotics have the potential to prevent and cure intestinal dysfunctions, inflammatory bowel disease, gastro-intestinal infections, and colon cancer. (Marteau P. & Boutron-Ruault, 2002) Probiotics, prebiotics, and the combination of prebiotics and probiotics are potent therapeutic tools designed to rectify this situation. Probiotics such as *Lactobacillus spp.* are more or less like stem cells utilized to replenish and rejuvenate the microbiome while prebiotics like fructose oligosaccharides are microbiome fertilizers akin to mineral supplements or energy nutrients aimed at promoting the proliferation of select microbes in the invisible organ. Synbiotics is a combination of both probiotics and prebiotics in a proper dosage aimed at remedying dysbiosis (Appanna, 2018).

Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota. If we have more emphasizes on nutrition, diet modification and restoring microbiota could prevent the occurrence of chronic diseases. The Synbiotic (Prebiotics and probiotics) and tryptophan rich diet could be beneficial for restoring of microbiota and also helpful in the absorption and metabolism of tryptophan may be helpful for prevention and treatment of various chronic diseases and it also overcome the adverse effect of medication which is used for the treatment of such diseases.

In the present study, we used a synbiotic combination i.e., prebiotic (*Allium sativum extract* and *Cucurbita maxima seed extract*) and probiotic containing sauerkraut and evaluated for its antiobesity and antidiabetic effects. *Pediococcus perosseous* probiotics is isolated and confirmed with different biochemical and genotypic analysis.

Materials and Methods

1.Prebiotics: The hydroalcoholic extract of Garlic and Pumpkin seed was obtained as gift sample with certificate of Analysis from Herbo Nutro life Noida India

2. Probiotic containing sauerkraut prepared and evaluated: *Pediococcus perosseous* probiotics is isolated and confirmed with different biochemical and genotypic analysis from sauerkraut.

2.1. Collection and authentication of Cabbage (*Brassica oleracea L. var. capitata*) Brassica oleracea: The cabbage (*Brassica Oleracea L. Var Capitata*) was purchased from local market of Latur, Maharashtra, India and authenticated by the expert in the Department of Botany, Dayanand Science College Latur, and Maharashtra, India. The plant Voucher specimens were preserved at the Department of Botany, Dayanand Science College Latur, Maharashtra, India

2.1.1 Preparation and fermentation of Sauerkraut for seven days: For the preparation of sauerkraut, the cabbage (*Brassica oleracea L.var.capitata*) was obtained from the local market of Latur. The damaged and defective cabbage heads were trimmed off and the cabbages were (shredded) with a sterile knife. The shredded cabbages were weighed 500 grams in three parts followed by the addition of 2.5 % salt (NaCl). The shredded cabbages (500 grams) and salt (12.5 grams) were placed in alternating layers in the wide mouth jars. A heavy weight board was placed over the mixture put in the jars and was pressed gently to squeeze out the juice. The jars were covered with sterile lids and incubated at 21°C to 24°C for 7 days for the fermentation. (Ram Kumar Pundir, 2010). After that, the physical property (weight loss) and chemical properties (pH) were analysed by pH paper (Fadhil et al., 2019).

2.1.2 Bacteria isolation and biochemical characterization from sauerkraut: LAB (Lactobacillus) were isolated on MRS agar (HiMedia, India) and incubated at 37 °C for 24–48 h. Single bacterial colonies that produced a white zone were selected according to morphological differences. Gram-staining, catalase tests and oxidase test was carried out for colonies. Only Gram-positive, Oxidase and catalase negative colonies were sub-cultured in MRS broth and kept in glycerol before experimental use.

2.13 Molecular characterization

1. 16 S rRNA gene sequencing and Phylogenetic analysis based on 16S rRNA gene: Pure viable microbial culture on Petri plates and broth sends towards the National Centre for Microbial Resource (NCMR) for molecular characterization.

3. Evaluation of effect of Synbiotic in High-fat diet fat and streptozotocin-induced diabetic Wistar rats.

3.1 Designing of Polyherbal colloidal solution for animal study:

On the basis of literature survey of all the ingredient of designed formulation we had selected the label dose of the drug for colloidal solution for *Cucurbita maxima* seed extract 200mg/kg, (Ebojele & Oraih, 2015), *Allium sativum* extract 400mg/kg (Sumiyoshi et al., 1984) and for sauerkraut 1.25ml/kg. (Krajka Kuźniak et al., 2011). By considering these label doses of drug, the colloidal solution designed and prepared for further study.

3.2 Oral glucose tolerance test in normal rat's animals and experimental setup: The oral glucose tolerance test (OGTT) is a usual assay to evaluate glucose tolerability and insulin resistance. The oral glucose tolerance test was performed in overnight fasted normal healthy rats. Rats were divided into three groups, each consisting of six rats were administered 0.9% (w/v) saline, metformin 250 mg/kg, and Polyherbal formulation (200 mg/kg), respectively. Glucose (3 g/kg) was fed 30 min after the administration of Polyherbal formulation. The blood samples were collected at 0, 30, 60, and 120 min by tail vein of rats by pricking and were immediately used for the estimation of blood glucose with a glucometer (Dhanabal et al.,2011).

3.3 Acute Toxicity Study and Dose Selection:

The acute toxicity study for polyherbal formulation (PHF) High Concentration was performed as procedure described in OECD guideline No.423 (AOT 423)

The toxicity study was performed on 03 female wistar rats. Before study rats were allowed access to only drinking water without food overnight. A single dose of PHF of 2000 mg/kg, p.o., administered to each rat and were observed individually and continuously for 30 min and periodically in 24 hr. to detect changes in the autonomic or behavioral responses as well as for tremors, convulsion, salivation, diarrhea, lethargy, sleep, coma etc. And animals were also further observed for any changes or mortality for the next14 days. Further, dose of 2000 mg/kg, per oral, administered to second set of 03 female rats.

Experimental protocol Approval: The experiment protocol was approved by Institutional animal Ethics Committee (IAEC) and care of animals was taken as per guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA).
Approval no: DYPCOP/IAEC/2021/01

a. Selection of dose:

As PHF was found safe at 2000 mg/kg, (OECD, 2002) for further pharmacological evaluation of it's both concentrations i.e., lower concentration (PHF-LC) and higher concentration (PHF-HC), 200mg/kg was selected.

b. Study Design:

3.4.1 Experimental animals: Wistar rats (180 to 300gm) of both sexes were procured from Laxmi Biofarm Pvt. Ltd., Pune and housed in polypropylene cages at 25±2°C temperature with 50 to 60% relative humidity and kept under 12:12 h light dark cycles. They were fed with standard pellet diet (Nutrivet Life Sciences, Pune) and drinking water ad libitum. All animals were allowed to acclimatize to laboratory conditions prior to experimentation. All procedures were carried out in day light period.

After one-week adaptation period, the rats were randomly divided into two groups: the control group normal diet (NG, n=12) and the HFD group (n=30). The compositions of the high fat diets are shown in table 6.21. Rats were fed on their corresponding diets for 8 weeks before injection of STZ. At the end of week 8, the normal diet control group were randomly divided into two groups (n=6) :(1) Normal Control-Normal Diet (NC-ND); (2) Streptozotocin- Normal Diet Control (35 mg STZ/kg -ND control) (Guo et al., 2018) the HFD-feeding rats were randomly divided into five groups (n=6): (1) HFD-Control, High fat diet control; (2) HFD – STZ Control (Placebo formulation); (3) Reference standard treatment control group ; (4) Polyherbal formulation low concentration Treated group (PHF-LC)) ; (5) Polyherbal formulation High concentration Treated group (PHF-HC). The rats were fasted overnight and given a single intraperitoneal injection of Streptozotocin (STZ) in a citrate buffer (pH 4) the next morning, and the NG rats received only the citrate buffer. The HFD feeding was continued for a more week (or normal diet for controls). Groups II to Group-VII continued HFD for two weeks after the treatment started with test formulation.

3.2.4 Biochemical parameters

On last day of the treatments, the blood samples were collected by retro orbital puncture and serum was separated by centrifugation at 4000 rpm for 15 min. The separated serum was used for biochemical parameter estimation. Such as, blood glucose, Triglycerides, total cholesterol, and HDL- cholesterol, HbA1c were analyzed from the serum by auto analyser (Friedewald et al., 1972). VLDL (very low-density lipoprotein)-cholesterol was calculated as: triglycerides/5; LDL (low density lipoprotein).

3.2.5 Histopatholtial analysis:

Sections of Liver and pancreatic tissue were fixed in 10% formalin. Tissues were trimmed to suitable size and thickness for proper penetration of reagents while processing. The tissues were processed routinely and fixed in paraffin. The sections of 3-5µm thickness were cut, stained with Hematoxylin and eosin stain and were examined microscopically

3.2.6 Statistical analysis:

All data are presented as the mean standard error of mean (SEM). Statistically analysed by one way ANOVA followed by Bonferroni multiple comparison test. A difference

with P<0.05 was considered significant. A difference with P<0.01 was considered extremely significant.

4. Results and Discussion:

1.Probiotic containing sauerkraut prepared and evaluated

1.1.Evaluation of Sauerkraut: As shown in the table 4.1 a significant decrease in the weight of fermented sauerkraut as Day 1 to Day 8 and the pH of the juice from sauerkraut at different stages of fermentation was determined by pH paper strips. As decrease in the pH with duration is the significant reflection of bacterial growth. These results confirm that fermented sauerkraut contains the bacterial growth.

Table no 1: Evaluation of sauerkraut

Day	Weight loss (excluding Weight of jars) in gram	pH
Day 1	**514±2.0**	**8.5**
Day 3	**512±2.0**	**6.5**
Day 5	**510±2.0**	**5.4**
Day 7	**505±2.0**	**3.9**
Day 8	**503±2.0**	**3.1**

1.2 Biochemical and molecular characterization of isolates from sauerkraut: Isolates were observed for colony morphology on MRS agar plates and the isolated colonies were white or creamish white, round, and flat or raised. Isolates was gram positive; catalase and oxidase negative signify that isolated microorganism is the gram-positive lactobacillus.

1.3 Molecular Identification by 16S rDNA Sequencing and Phylogenetic Analysis: The isolate strain no W/P/003, identified *as Pediococcus pentosaceus* DSM20336(T) with accession no. JQBF01000022, proved to have excellent probiotic properties. The bacterial sample(s) identification based on 16S rRNA, the identification report was generated using EzBioCloud Database at NCMR Pune. And **Sequence Text (in FASTA format font: courier new 10)** was found to be TGCTCAGGATGAACGCTGGCGGCGTGCCTAAT.

2. Evaluation of effect of Synbiotic in High-fat diet fat and streptozotocin-induced diabetic Wistar rats.

2.1 Acute toxicity study: A single dose of PHF of 2000 mg/kg, p.o., administered to each rat and were observed individually and continuously for 30 min and periodically in 24 hr. to detect changes in the autonomic or behavioral responses as well as for tremors, convulsion, salivation, diarrhea, lethargy, sleep, coma etc. and were further observed for any changes or mortality for the next14 days. Further, dose of 2000 mg/kg, per oral, administered to second set of 03 female rats. No signs of changes in autonomic or behavioral responses or mortality were observed during next 14 days of study. As PHF

was found safe at 2000 mg/kg, for further pharmacological evaluation of its both concentrations i.e., lower concentration (PHF-LC) and higher concentration (PHF-HC), 200mg/kg was selected.

2.2 Oral glucose tolerance study: Oral Glucose Tolerance Test, the blood samples were analysed for glucose content at - 0, 30, 60, 120 and 180 minutes, respectively. The blood glucose levels of polyherbal formulation (200 mg/kg) treated groups were not significant with control and metformin treated at a -0, 30, 60,120 and 180 minutes. The blood glucose levels at 60 minutes in polyherbal formulation and metformin treated lower than normal group. (Table 2)

Table No 2 The end of oral glucose tolerance test (OGTT) result

Treatment	Blood Glucose Level (mg/dL) Mean ± SD				
	0 hrs	30 mins	60 mins	120 mins	180 mins
Normal Group	86.84±0.99	128±1.68	115.17±1.48	103.5±2.26	94.84±1.17
Positive control [Metformin 500mg/kg, p. o}	87.5±1.05ns	76.17±1.17***	71±1.27***	63.5±1.88***	62±2.45***
Poly herbal formulation High Concentration	87±0.9ns	123.34±2.17***	104.84±1.17***	96.17±1.17***	87.34±1.76***

All values are expressed as mean ± SD, n=6. All data are subjected to one Way ANOVA followed by Bonferroni's multiple comparisons test. Test Drug and Standard Drug Treated are compared with Control group *p<0.05, **p<0.01, *** p<0.001, NS: Not Significant

Table 3. The effect on body weight of polyherbal formulation in two different concentration and standard on High Fat Diet &

Week	GR-I Normal Contorl (NC-ND) (gm)	GR-II STZ Control (gm)	GR-III HFD Control (gm)	GR-IV Diabetic (HFD-STZ) Control (gm)	GR-V- Reference Standard (Metformin) Treated (gm)	GR-VI-PHF-LC-200mg /kg (gm)	GR-VII- PHF-HC-200mg /kg (gm)
1	252 ± 12.88	216 ± 17.66	255 ± 24.29	229 ± 14.29	225 ± 23.45	238 ± 16.05	233 ± 16.02
2	255 ± 14.14	222 ± 17.24	264 ± 32.93	243 ± 16.66	238 ± 20.17	253 ± 14.05	245 ± 15.16
3	262 ± 13.65	229 ± 18.35	297 ± 32.04	259 ± 17.43	249 ± 21.54	263 ± 13.32	258 ± 11.25
4	269 ± 15.94	238 ± 17.26	318 ± 33.12$^{\#}$	282 ± 20.66	264 ± 22.45	278 ± 17.22	268 ± 10.80
5	275 ± 17.32	242 ± 18.63	317 ± 33.12	293 ± 18.43	274 ± 23.75	293 ± 17.51	278 ± 12.14
6	283 ± 18.91	245 ± 19.36	332 ± 31.62 $^{\#}$	308 ± 17.82	283 ± 23.38	297 ±.10.33	280 ± 10.09
7	292 ± 19.41	204 ± 19.24$^{\#\#\#}$	340 ± 30.77 $^{\#}$	276 ± 16.86	275 ± 20.0	272 ± 9.83	262 ± 11.69
8	301 ± 20.10	178 ± 10.67 $^{\#\#\#}$	348 ± 27.39$^{\#}$	258 ± 14.32	265 ± 16.43	250 ± 6.32	243 ± 13.32

Streptozotocin induced diabetes in wistar rats

Values are expressed as (Mean ± SEM), n=6, # = p<0.05, ##= p<0.01, ### = p<0.001 when compared to normal control group. Statistically analysed by one way ANOVA followed by Bonferroni multiple comparison test.

2.3 Blood Glucose level: Effect of Polyherbal formulation (PHF) on fasting blood glucose level in rats at week 8 **Table No:3. Effect of Polyherbal formulation (PHF) on fasting blood glucose level in rats at week 8**

GR-I NC-ND	**GR-II STZ-ND**	**GR-III HFD Control**	**GR-IV Diabetic (HFD-STZ) Control**	**GR-V-Metformin Treated**	**GR-VI-PHF-LC-200mg /kg**	**GR-VII-PHF-HC-200mg /kg**
73.5 ± 2.65	289.16 ± 10.79 ###	139.66 ± 12.49 ##	344.5 ± 16.27 ###	153.33 ± 7.11***	278.5 ± 11.98**	239.33 ± 13.64 ***

Values are expressed as (Mean ± SEM), n=6, # = p<0.05, ##= p<0.01, ### = p<0.001 when compared to normal control group *= p<0.05, **=p<0.01, *** = p<0.001 when compared to Diabetic (HFD-STZ) control group (Gr-IV) statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test

Results indicate that there was significant (p<0.001) increase in blood glucose level group-II (STZ-ND), (p<0.01), in group-III (HFD-control) and (p<0.001) in group-IV Diabetic (HFD-STZ) control group when compared to normal control (NC-ND) group.

PHF lower concentration (PHF-LC) and PHF high concentration (PHF-HC) at 200mg/kg reduced blood glucose significantly (p<0.01) & (p<0.001) respectively when compared to Diabetic (HFD-STZ) control group. Reference standard metformin was most effectively (p<0.001) reducing the blood glucose level when compared to Diabetic (HFD-STZ) control group.

2.4 Lipid Profile and HbA1C:

Effect of Polyherbal formulation (PHF) on Lipid Profile and HbA1C in rats at week 8

Table No 4. Effect of Polyherbal formulation (PHF) on Lipid Profile and HbA1C in rats at week 8

Sr. No.	Treatment / Groups	Parameters				
		Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Glycosylated Hemoglobin (HbA1C-%)
1	GR-I Normal Control-(NC-ND)	68.78 ± 2.79	87.25 ± 7.1	28.85 ± 3.12	24.33 ± 1.5	4.31 ± 0.12
2	GR-II STZ-ND	108.55 ± 6.31$^{###}$	106.48 ± 6.99^{NS}	38.06 ± 2.2 ^{NS}	19.30 ± 2.25 ^{NS}	10.68 ± 0.62$^{###}$
3	GR-III HFD Control	147.08 ± 5.64$^{###}$	186.08 ± 5.84$^{###}$	59.70 ± 4.75$^{##}$	17.91 ± 1.51 ^{NS}	6.76 ± 0.41$^{##}$
4	GR-IV Diabetic (HFD-STZ) Control	159.45 ± 8.26 $^{###}$	230.16 ± 12.92^{c}	109.46 ± 6.71$^{###}$	11.73 ± 1.37$^{###}$	12.53 ± 0.35$^{###}$
5	GR-V- Metformin Treated	104.33 ± 5.63***	138.66 ± 7.48***	79.96 ± 7.06**	21.733 ± 1.75**	7.55 ± 0.35***
6	GR-VI- PHF-LC-200mg /kg	128.11 ± 4.88*	192.25 ± 12.85^{NS}	95.45 ± 5.6 ^{NS}	20.43 ± 2.22*	11.8 ± 0.51 ^{NS}
7	GR-VII- PHF-HC-200mg /kg	112.55 ± 6.66***	160.5 ± 10.64**	84.53 ± 4.63*	20.9 ± 1.61*	9.6 ± 0.48***

Values are expressed as (Mean ± SEM), n=6, # = p<0.05, ##= p<0.01, ### = p<0.001 when compared to normal control group *= p<0.05, **=p<0.01, *** = p<0.001 when compared to Diabetic (HFD-STZ) control group (Gr-IV) statistically analysed by one way ANOVA followed by Bonferroni multiple comparison test.

2.5 Effect of Polyherbal formulation (PHF) on the weights of kidneys, liver and pancreas:

All the rats were fasted overnight and sacrificed under deep anesthesia. The liver, kidney, and pancreas along were dissected, dried by tissue papers and weighed and was statistically analyzed

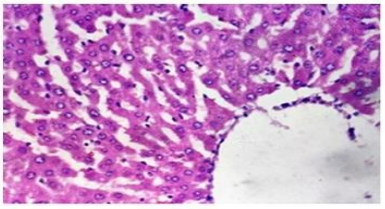
Table 4: Effect of Polyherbal formulation (PHF) on the weights of kidneys, liver and pancreas:

Sr. No.	Treatment / Groups	Liver Weight (gm) (Mean ± SEM)	Kidney Weight (gm) (Mean ± SEM)	Pancreas Weight (gm) (Mean ± SEM)
1	GR-I Normal Control-(NC-ND)	5.57 ± 0.18	0.52 ± 0.014	0.27 ± 0.015
2	GR-II STZ-ND	3.76 ± 0.24$^{\#\#}$	0.51 ± 0.019	0.18 ± 0.011$^{\#\#}$
3	GR-III HFD Control	7.20 ± 0.19 $^{\#}$	0.59 ± 0.012	0.23 ± 0.017
4	GR-IV Diabetic (HFD-STZ) Control	4.88 ± 0.50	0.43 ± 0.018$^{\#}$	0.21 ± 0.018
5	GR-V- Metformin Treated	5.64 ± 0.23	0.53 ± 0.013**	0.25 ± 0.007
6	GR-VI- PHF-LC-200mg /kg	6.41 ± 0.42	0.52 ± 0.02*	0.19 ± 0.01
7.	GR-VII- PHF-HC-200mg /kg	5.90 ± 0.37	0.52 ± 0.019*	0.23 ± 0.017

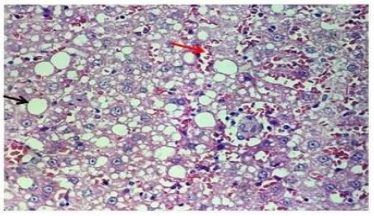
Values are expressed as (Mean ± SEM), n=6, # = $p<0.05$, ##= $p<0.01$, ### = $p<0.001$ when compared to normal control group *= $p<0.05$, **=$p<0.01$, *** = $p<0.001$ when compared to Diabetic (HFD-STZ) control group (Gr-IV) Statistically analyzed by one way ANOVA followed by Bonferroni multiple comparison test.

2.6 Effect of Polyherbal formulation (PHF) on the histopathology of the liver and pancreas:

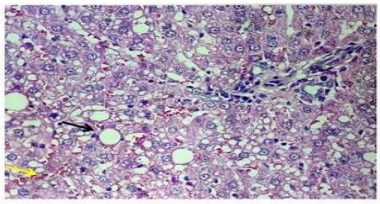
All the animals were euthanized by CO2 asphyxiation, for collection of the liver and pancreas. Organs were collected in 10% neutral buffered formalin. Tissues were trimmed to a suitable size and thickness for proper penetration of reagents while processing. The tissues were processed routinely and embedded in paraffin. The sections of 3-5µm thickness were cut, stained with Hematoxylin and eosin stain, and examined microscopically.



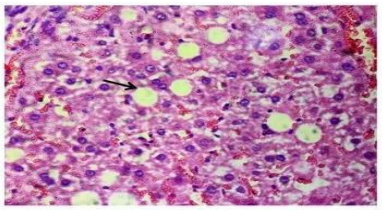
Normal liver 40X, H&E
a) GR-I Normal Control-(NC-ND)



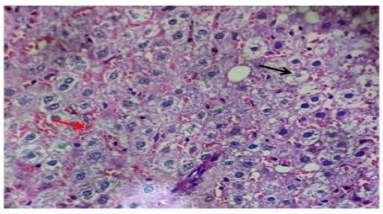
Moderate multifocal vacuolar degeneration of various sizes of hepatocytes (Black arrow), Mild Congestion in sinusoidal spaces, (Red arrow) architectural details are lost, Mild degenerative of hepatocytes,40X, H&E
b)GR-II STZ-ND



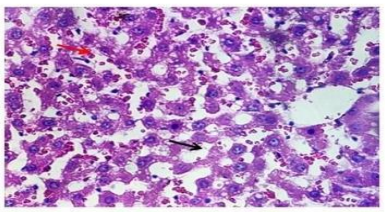
Moderate multifocal vacuolar degeneration of hepatocytes (Black arrow), Minimal infiltration of polymorphonuclear cells, Mild focal congestion in sinusoidal spaces (yellow arrow), Mild degenerative of hepatocytes, 40X, H&E
c) GR-III HFD Control



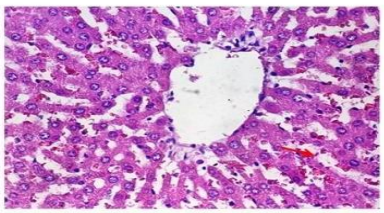
Mild focal vacuolar degeneration with increase size of vacuoles (Black arrow), Mild focal congestion in sinusoidal spaces (Red arrow), Minimal degeneration of hepatocytes,40X, H&E
d) GR-IV Diabetic (HFD-STZ) Control



Minimal focal vacuolar degeneration of hepatocytes (Black arrow), Mild focal congestion in sinusoidal spaces (Red arrow), Minimal degeneration of hepatocytes,40X, H&E
e) GR-V- Reference Std. (Metformin) Treated



Mild focal increased sinusoidal spaces (Black arrow), Minimal focal congestion in sinusoidal spaces (Red arrow), arcitectural details are maintained 40X, H & E
f)GR-VI- PHF-LC-200mg /kg



Minimal focal congestion in sinusoidal spaces (Red arrow), arcitectural details are maintained,40X, H&E. g) GR-VII- PHF-HC-200mg /kg

Fig 1: Effect of Polyherbal formulation (PHF) on the histopathology of liver

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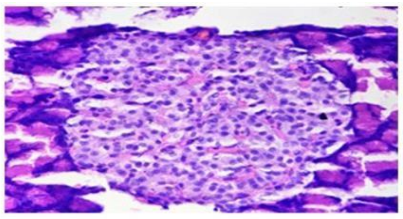
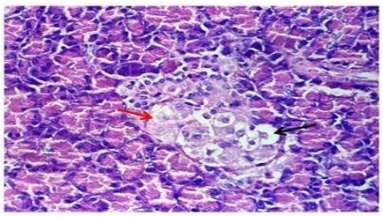
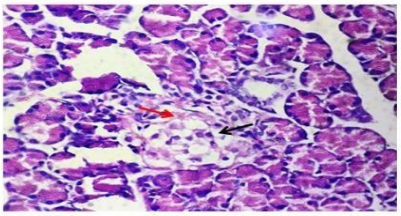


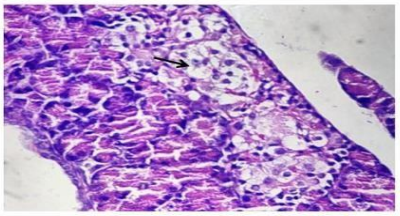
Normal pancreas 40X, H&E
a)GR-I Normal Control-(NC-ND)



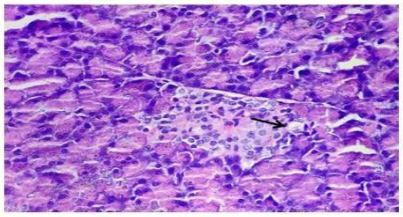
Moderate multifocal vacuolar degeneration of β- cells in Islets (Black arrow), Minimal β-cells necrosis (Red arrow), Minimal focal infiltration of
polymorphonuclear cells, 40X, H&E
b) GR-II STZ-ND



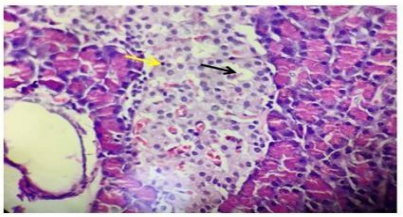
Mild multifocal vacuolar degeneration of β-cells in Islets (Black arrow), Mild β-cells necrosis (Red arrow), 40X, H&E.
c) GR-III HFD Control



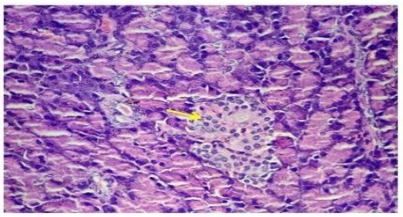
Mild focal vacuolar degeneration of acinar cells (Black arrow), 40X, H&E.
d) GR-IV Diabetic (HFD-STZ) Control



Minimal focal vacuolar degeneration of β-cells in Islets (Black arrow), 40X, H &E
e) GR-V- Reference Std. (Metformin) Treated



Minimal focal vacuolar degeneration of β-cells in Islets (Black arrow), Minimal focal infiltration of polymorphonuclear cells (Yellow arrow) congestion 40X, H & E
f)GR-VI- PHF-LC-200mg /kg



Minimal focal infiltration of polymorphonuclear cells (Yellow arrow) 40X, H&E.
a) GR-VII- PHF-HC-200mg /kg
7.22 Effect of Polyherbal formulation (PHF) on the histopathology of Pancreas

Fig 2: Effect of Polyherbal formulation (PHF) on the histopathology of Pancreas

Discussion:

In this study, we evaluated the efficacy of these Synbiotic in alleviating the effects of HFD- and STZ induced type 2 diabetes. High-fat diet fed for three or more weeks is said to induce insulin resistance and initiate a low-grade inflammatory tone in important organs of the body making the host highly susceptible to attaining type 2 diabetes. Conversely, HFD coupled with a low dose of STZ is said to mimic the metabolic characteristics of type 2 diabetes and is a novel model for anti-diabetic studies. (Magalhães Et Al., 2019). This is the first study to combine a novel formulation using Sauerkraut, Allium sativum and Cucurbita maxima as a probiotics and prebiotics. The administration of a dose of Streptozotocin (35 mg/kg) after 2 weeks of a dietary schedule of HFD to rats is based on an earlier report by (Srinivasan et al., 2005) HFD rats may be susceptible to insulin resistance because the receptor cells are blocked by fat deposits, and the diabetic action of a low dose of STZ is enhanced. Hence, it has been recognized by several researcher that the synergistic effect of HFD and a low dose of Streptozotocin may reflect the development of an ideal model for noninsulin dependent diabetes mellitus (Skovso, 2014).

As per the extensive literature survey it was clearly indicates the HFD and STZ in combination is responsible for alteration in gut microbiota which may lead to various chronic diseases such as obesity and diabetes (Liu et al., 2019). Rats that consumed a high-fat diet exhibited increased plasma glucose levels and plasma lipid levels, including cholesterol and triglyceride levels. The saturated fats present in the high-fat diet are responsible for the increase in the glucose and lipid profiles (Timmers et al., 2011).

According to previous studies, rat fed a high-fat diet display increased serum glucose concentrations. Rats fed a high-fat diet also developed glucose intolerance and were unable to properly utilize glucose to establish homeostasis after a glucose challenge (Akiyama et al., 1996). In fact, the gram-negative bacterial cell wall contains lipopolysaccharide (LPS), which may penetrate the gut due to the leakiness of intestinal mucosa caused by a high fat content in the diet (Cani et al., 2008). Researchers have postulated that bacterial LPS may cause endotoxemia and inflammation (Naito et al., 2011). Intestinal permeability may also facilitate the entry of bacterial fragments into the body and subsequent interaction with the Toll-like receptor to activate innate and adaptive immunity and cause hyperglycemia and insulin resistance (Amar et al., 2011).

However, Lactobacilli species are capable of strengthening the epithelial barrier and may potentially prevent LPS-mediated inflammation and hyperglycemia26. Yogurt supplementation improved the glucose utilization in this experiment, as evidenced by the results from the OGTT, and these results are supported by a previous experiment (Hummel et al., 2012). Based on the previous study, Polyherbal formulation of sauerkraut, Allium sativum and Cucurbita maxima in lower concentration (PHF-LC) and Higher concentration (PHF-HC) used for the evaluate the antiobesity and hypoglycemic effect in HFD and STZ induced diabetes. Previous research suggests the reduction of inflammatory therapy on β-cells in pancreas contributed the synthesis of proinsulin to insulin by increased cell mass and

insulin sensitivity (Hariom et al.). indicated that, the probiotic yogurt supplemented diet significantly delayed the onset of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in high fructose-induced diabetics rats, indicating a lower risk of diabetes and its complications.

The significant elevation of body weight and blood glucose level in type 2 diabetic rats when compared to normal control diet rat could be attributed to the dietary regimen of HFD and STZ administration. However, the hyperglycemic state in type 2 diabetic control rats were gradually suppressed in groups treated with metformin and PHF-LC and PHF-HC.

Conclusion:

Prevention and treatment of diabetes without any side effects is still a challenge to the medical system. However, natural supplements are widely used around the world to treat diabetes, but medical research does not support their effectiveness. Therefore, the search for natural supplement from fermented food containing prebiotics and probiotics is being intensified probably because of its fewer side effects, readily availability and low cost with preventive approach.

In this study Synbiotic combination in higher concentration shows significant lipid lowering and glucose lowering property with normal structure of liver and pancreas by comparative study of histopathology. In the present study we used the common fermented products and vegetables, and they have significant role for the treatment of diseases. If we prepare the optimum formulation of the prebiotics, probiotics combination and adding these products in regular diet could be the prophylactic approach for the treatment of various chronic disease.

Conflicts of Interest: The authors declare no conflict of interest.

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