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Dose Dependent Effect of Probiotic Supplemented Herbal Wine from *Tinospora Cordifolia* in Metabolic Syndrome: An Experimental Study

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Abstract

Metabolic syndrome a lifestyle disease which encompasses obesity, dyslipidemia, hyperglycemia and chronic inflammation in metabolic tissue, diet and gut microbiota play a prodigious role in its initiation and progression. Bio-interventions employing probiotic and wine offer an alternate nutritional approach towards attenuating the progression of metabolic syndrome. The present study aimed to evaluate the protective efficacy of low, moderate and high dose of probiotic supplemented herbal wine in an experimental metabolic syndrome. It was observed that though the administration of low, moderate and high dose of probiotic supplemented herbal wine for 12 weeks to Sprague Dawley rats fed with high fat diet ameliorated the anthropometric parameters but low to moderate dose showed maximum reduction in weight gain, abdominal circumference, BMI, Lee's index, and visceral fat deposition compared with high fat diet fed animals. Additionally, both low and moderate dose of probiotic supplemented herbal wine exhibited improved glucose tolerance, liver biomarkers and restored the histoarchitechture of liver, colon and adipose tissue compared with high fat diet fed animals. The study highlights the prophylactic potential of probiotic supplemented herbal wine in experimental metabolic syndrome model and revealed that amongst all three doses of probiotic supplemented herbal wine low and moderate dose were found be effective and more promising than high dose in improving metabolic dysfunctions and may be employed as functional foods but needs further validation.

Keywords: Anthropometric, Biomarker,Diabetes, Dose, High fat diet, Herbal wine, Functional food, Metabolic syndrome, Obesity, Probiotic supplemented herbal wine

Introduction

Inclination towards fast food culture and sedentary life has led to the enhanced prevalence of metabolic syndrome (MetS). MetS is a complex disorder caused by cluster of interrelated factors that enhances the risk of cardiovascular disease and type 2diabetes and has emerged as public health challenge affecting about one quarter of the world's population[1-3]. Accumulating evidences suggest that regular consumption of high calorie diet induce dysbiosis leading to development of insulin resistance, obesity and other hallmarks of metabolic syndrome[4-5]. However, due to high concern of people towards health has further prompted the interest towards development of health promoting foods or physiologically active food components known as functional foods [6-8]. Functional foods encompass fiber enriched grains, fruits, vegetables, phytochemicals, probiotics, beverages such as tea, coffee and wine and are known to modulate various biological activities such as absorption, immune-stimulation,diabetes, dyslipidemia, cholesterolemiaand can serve as a novel strategy for the management of metabolic syndrome [9-11].

Probiotics, the future biotherapeutic agents are live microorganisms that when administrated in adequate amounts, confer a health benefit on the host[12].Moreover, both experimental and clinical studies have demonstrated the alleviating potential of probiotic in various diseases such as obesity, hepatic steatosis, insulin resistance and gastrointestinal disorder, colon-rectal cancer, giardiasis[13-15]. Food that contains viable probiotic microorganism not only improves the nutritional value of food but also had tremendous health benefits such as improve intestinal microbiota balance, anti-obesity and anti-diabetic. More specifically, dairy fermented products such as yogurts, cheese and fermented sour milk remains at the forefront of probiotic food that has been traditionally considered as the best carrier for probiotics[16]. However, the increasing health concern of lactose intolerance, milk protein allergy, high cholesterol content and high amount of saturated fatty acid of dairy based foods are resulting into shift towards non-dairy foods such as probiotic fermented cereals, fruits and vegetable beverages[17].

Among various functional beverage, wine is the most researched and validated beverage. Wine is generally produced from grapes, along with other fruits such as mango, apple, berries. Apart from fruits, herbal wines are also emerging as better choice being prepared from amla, ginger, mentha arvensis etc because of their medicinal value that has prompted the scientific interest to explore various other herbs for the production of functional wine and endorse their pharmaceutical benefits[18-21].

Tinospora cordifolia (Giloy),the tremendously used herbs in ayurvedic medicineconstitute innumerable biologically active compounds that have been reported to have prophylactic and therapeutic potential in both experimental and clinical studies to combat different ailments such as hepatoprotective effect, glucose tolerance, anticancer, antidiabetic,immune modulatory potential [22-24]. Therefore, an attempt has been made to prepare probiotic supplemented herbal wine from giloy after statistical optimization for various fermentation processesvis-a- vis to validate the beneficial potential experimentally.Thus, the present study was designed with an aim to find out the effective dose of prepared probiotic supplemented herbal wine that could attenuate the effect of experimentally induced metabolic syndrome particularly w.r.t anthropometric parameters, adiposity markers, fecal LAB count, oral glucose tolerance test, serum lipid profile and histological alterations in liver, colon and adipose tissue.

Materials and methods

Bacterial Strains

Bacterial strains *Lactobacillus rhamnosus*GG MTCC #1408, *Lactobacillus plantarum* MTCC #1407, and yeast strain *Saccharomyces cerevisiae* MTCC #786 were procured from Microbial type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India. *Lactobacillus acidophilus* NCDC #15 was procured from National Dairy research institute, Karnal, India. Four Lactic acid bacteria(LAB) (*Pediococcusacidilactici* BNS5B, *Lactobacillus pentosus* GSSK2, *Lactobacillus fermentum* B3GS5, *Lactobacillus fermentum* PUM) isolated from different sources and well characterized for their potential probiotic attributes in our research laboratory were employed. All the probiotic strains and other bacterial strains were grown in De Man Rogosa and Sharpe (MRS) and Luria broth (LB) respectively and were preserved in 50% glycerol and stored at -20˚C while yeast strain was grown in glucose yeast broth (GYE, 0.3%yeast extract, 0.3% malt extract, 0.5% peptone, 1% glucose) and maintained on Potato dextrose agar (PDA) slants and stored at 4˚C.

Preparation of probiotic supplemented herbal wine

Processing of *Tinospora cordifolia* (giloy) stem

Giloy stem was procured from local nursery of Chandigarh, India, were properly washed, cut into small pieces and added to distilled water containing flask in a ratio of 10% (w/v), autoclaved (referred as stemaqueous extract) and was used for further experiments.

Inoculum preparation

*Saccharomyces cerevisae*was grown in 25ml of sterilized GYE broth at 30˚C for 24h on a rotary shaker. The pre-inoculum was prepared by cold centrifuging the cells at 4000g for 15 min, washed twice and suspended (10^8 cells/mL) in normal saline. The inoculum was prepared by transferring 10ml of pre-inoculum to 100ml of stem aqueous extract, the total soluble solid (TSS) was adjusted at 5˚Brix with cane sugar followed by incubation at 30˚C on a rotary shaker for 24h.

Fermentation of *Tinospora cordifolia* (giloy) stem

For wine production, 100ml giloy stem aqueous extract added in 250ml flasks and supplemented with cane sugar to adjust TSS to 20 ˚brix and pH was adjusted to 4 using citric acid/sodium bicarbonate, followed by the addition 100ppm of potassium metabisulphate,7.9gm ammonium dihydrogen phosphate, 778mg KH_2PO_4, 197mg $MgSO_4$, 900mg thiamine, 800mg isoleucine (referred as production media). Thereafter, it was inoculated with 10% (v/v) inoculum of *S.cerevisiae* (10^8 CFU/ml) and kept for fermentation in incubator at 30±2˚C. The content of flask was mixed 2-3 times a day and the progress in fermentation was monitored at a regular interval of 24h by analyzing TSS, pH, ethanol [25], total phenolic compounds [26] and antioxidant capacity [27]

Supplementation of prepared herbal wine with probiotic strains

All the probiotic culture were grown in MRS broth at 37˚C for 18h, cold centrifuged at10000rpm for 10 min, washed and suspended (1× 10^9 CFU/ml) in prepared wines and kept in refrigeration. Probiotic viability was monitored at regular interval of 4 days for a period of 5weeks by spread plate method and probiotic culture showing maximum viability was selected for supplementation of wine.

To standardize the dose of probiotic supplemented herbal wine (PSHW) for metabolic syndrome in Sprague Dawley rats

Animals

Male Sprague Dawley (SD) rats (150-180g) were procured from the Central Animal House, Panjab University, Chandigarh, India. These were housed in polypropylene cages (3 animals per cage) with a wire mesh top and hygienic bed of husk (changed regularly) in room with standard conditions i.e., 12 h light/dark cycle, temperature (22± 2°C) and humidity (55±5%). Animals were acclimatized for 7–10 days and provided with standard pellet diet (Ashirwad Industries, Chandigarh, India) and water ad libitum. Care, use and disposal of animals were done in accordance with the guidelines of the Institutional Animals Ethical Committee (IAEC), Chandigarh and approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals **(PU/45/99/CPCSEA/IAEC/2021/536).**

Preparation of high fat diet (HFD)

Standard pellet diet (6% calories derived from fat) comprised of corn starch (45.5%), maltodextrin (3.5%), cellulose (4.7%), dextrin (10%), soybean oil (6.3%), casein (25%), vitamin and mineral mix (4.5%), and DL-methionine (0.5%) [28]. HFD (60% calories derived from fat) was prepared in-house as per Khanna et al. 2020 (15). HFD comprised of powdered standard diet (36.5% w/w), milk casein (25.0% w/w), animal lard (32.0% w/w), vitamin and mineral mix (6.0% w/w), yeast extract (0.1% w/w), DL-methionine (0.3% w/w) and sodium chloride (0.1% w/w).

Experimental design

In order to standardize the dose of **probiotic supplemented herbal wine (PSHW)** animals were divided into five groups, each group comprised of 6 animals and treated as follows.

- **Group I (Control):** Rats were fed with normal pellet diet for 12 weeks and were provided water *ad libitum*
- **Group II (HFD):** Rats were fed with high fat diet for 12 weeks
- **Group III Lowest dose (200ml/70kg) of PSHW (LPSHW+HFD):** Rats were administered orally with a single dose of 200ml/70kg PSHW and HFD daily for a period of 12 weeks
- **Group IV Medium dose (400ml/70kg) of PSHW (MPSHW+HFD):** Rats were administered orally with a single dose of 400ml/70kg PSHW and HFD daily for a period of 12 weeks
- **Group V Highest dose (600ml/70kg) of PSHW (HPSHW+HFD):** Rats were administered orally with a single dose of 600ml/70kg PSHW and HFD daily for a period of 12 weeks

Follow up of the animals

Animals belonging to group III, IV and V were fed orally with a single dose of PSHW daily via orogastric gavage for 12 weeks and were given HFD ad libitum. During the treatment body weight, fecal lactic acid and blood glucose were monitored weekly. However, after completion of 12 weeks, animals were sacrificed by injecting ketamine hydrochloride (80 mg/kg) intraperitoneally followed by cervical dislocation. Thereafter, blood was drawn via cardiac puncture to assess serum biochemical parameters i.e., lipid profile and liver function test vis- a vis histopathological alteration of liver, adipose tissue (epididymal and retroperitoneal) and colon.

Evaluation of anthropometric parameters and adiposity markers

Animals belonging to all the groups were assessed for body weight, weight gain, Lee's index abdominal circumference and Body Mass Index (BMI). The body weight of animals was recorded weekly on ordinary balance (SD-300, S.D fine chemicals Ltd, Chandigarh, India) throughout the experiment. Abdominal circumference was measured at the beginning and at the end of study using ordinary measuring tape [29]

Weight gain was calculated as (final body weight- initial body weight), Lee's Index was calculated as the cube root of body weight (g) /naso-anal length (cm) and Body Mass Index (BMI) was calculated as body weight (g)/length^2 (cm^2) at the end of the experiment, one day prior to sacrifice [30]

Feed intake of animals belonging to all the groups was recorded twice a month and was calculated by subtracting the amount of residual food in each cage from the weighed amount of food provided on the previous day (g/day) and represented as average feed intake (g/day/ rat) by dividing the feed intake by total number of animals. Liver and adipose tissue (epididymal and retroperitoneal) were weighed using ordinary balance.

Fecal Lactic acid bacteria count

In order to assess the effect of HFD induced metabolic syndrome on the LAB count in the colon, freshly voided fecal material (0.5 g/animal) was taken from each group once a week, was homogenized in normal saline, serially diluted and plated on MRS agar. The plates were incubated at 37°C for 48 h and colony forming units (CFU) were recorded [31].

Fecal lipids were estimated using classical phase separation-based method [32]. Briefly, 200 mg of dry feces was taken in a centrifuge tube and 3 mL of chloroform–methanol mixture (2:1, v/v) was added, vortexed for 1 min and centrifuged at 3000g for 10 min at 25°C. The chloroform phase containing the lipid fraction was collected in a fresh tube and after complete drying, total lipids were estimated as per Fringes and Dunn [33]

Blood glucose and oral glucose tolerance test

The fasting blood glucose levels of animals were recorded weekly using glucometer (Freestyle Optium Glucometer, Abbott diabetes care Ltd., Oxon, UK). Oral glucose tolerance test (OGTT) was performed one day prior to sacrifice. Briefly, animals were fasted for 6 h and blood glucose concentration was measured. Animals were then administered D-glucose (2 g/kg) orally and glucose concentration was determined at an interval of 15, 30, 60, 90, and 120 minutes respectively, via tail snip method using glucometer [30]. Area under the concentration-time curve (AUC) was calculated using GraphPad PRISM 7 software.

Analysis of serum lipid profile

Blood (0.5ml) from animals was collected in a microcentrifuge tube through cardiac puncture and serum was prepared. From the serum, both liver function test (Bilirubin, aspartate transaminase (AST), and alanine transaminase (ALT) and lipid profile (Total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol) were estimated using autoanalyser (Sysmex XP-100).

Histological analysis

For cellular and morphological changes, a portion of liver, colon and adipose tissue were employed [15]. The 10% formalin- fixed tissues were washed and dehydrated for 1-2hours in an ascending series of ethyl alcohol i.e., 30%, 50%, 70%, 90% and 100%. Dehydrated tissues were transferred in a 1:1 combination of benzene and absolute alcohol for 1 hour, followed by in pure benzene for half an hour, then in a 1:1

mixture of benzene and wax for another 1 hour, and finally in wax for 6 hours with a 3hour gap between changes. 6-micron thick sections of tissues were cut, embedded, placed on separate clean glass microscope slide, stained with hematoxylin and eosin (H&E) stain, viewed by light microscopy and photomicrographs were obtained using Nikon Eclipse 80i (Nikon, Kawasaki, Japan)

Statistical analysis

Results were analyzed statistically and expressed as mean ± standard deviation (SD). One way ANOVA followed by Tukey's multiple comparison test was applied to assess intergroup variations however for body weight, OGTT and LAB count, repeated measures ANOVA followed by Tukey's multiple comparison test was applied to analyze each treatment along time as well as intergroup variations at different time points using GraphPad PRISM software version 7 (GraphPad Software Inc., La Jolla, CA, USA). The statistical significance was defined as p and calculated at $p<0.01$, $p<0.05$.

Results

Light yellow colored wine was obtained after fermentation of giloy stem containing ethanol (11±0.08% v/v), TSS (3˚Brix), total phenolic (784±1.2 µg/ml), pH (3.3±0.04) and antioxidant potential (157±0.4µM/ml). Further, it was found that among seven probiotics supplemented in prepared herbal wine, *L.pentosus*GSSK2 exhibited highest survival rate of 88.6% for a period of 4 weeks followed by *P.acidilactici*BNS5B (85.2%) and *L.rhamnosus*GG (58%) respectively (Fig 1). Therefore, probiotic supplemented herbal wine was prepared with *L.pentosus*GSSK2 and was assessed for its modulating potential in experimental metabolic syndrome.

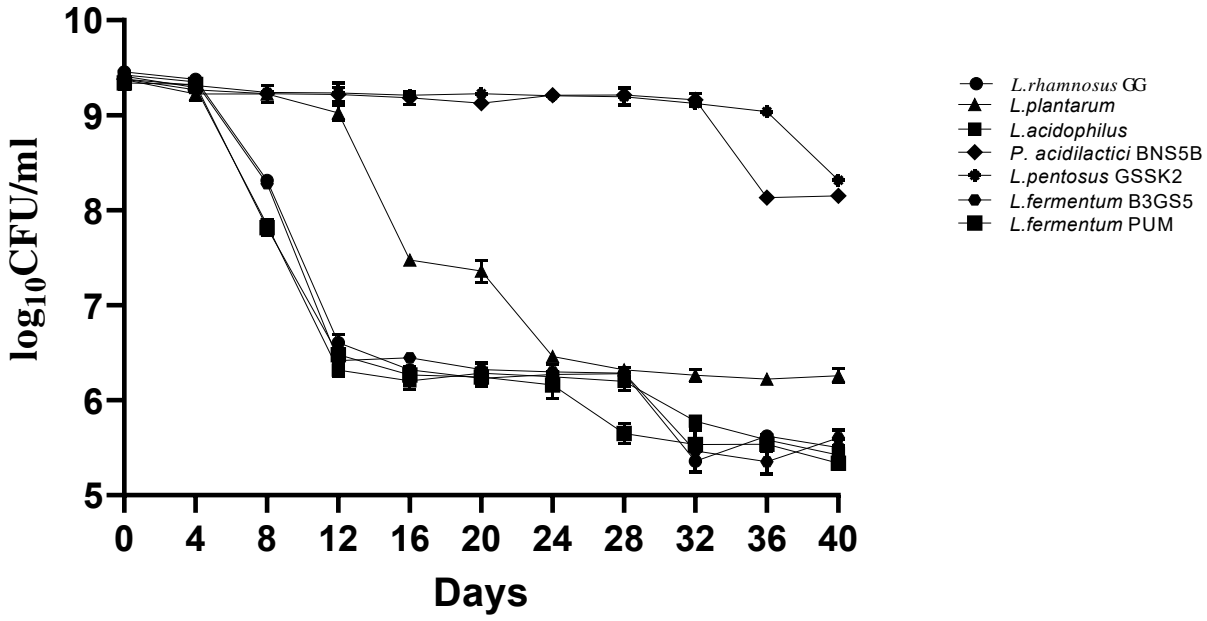

Fig.1Survival of different probiotic strains in the prepared giloy stem wine. Results are expressed as Mean ± standard deviation in three independent experiments.

Effect of probiotic supplemented herbal wine on anthropometric parameters and adiposity

It was observed that though supplementation of different doses of PSHW to HFD animals (Group III, IV, V) led to gradual increase in body weight but was significantly ($p<0.05$) less at each point of observation compared with HFD animals (Group II). More specifically, it was found that animals fed with different doses of PSHW did not show any difference in percent weight gain but the percent gain in body weight was significantly ($p<0.05$) less in animals belonging to LPSHW+HFD (44%; Group III), MPSHW+HFD (45%; Group IV) and HPSHW+HFD (46%; Group V) compared with 77% increase body weight of HFD (Group II) animals (Fig 2a, b).

It was also observed that feed intake of animals belonging to PSHW (Group III, IV, V) was almost same as that of HFD animals (Group II) and control animals (Group I; Fig 2c) but Lee's index, BMI and change in abdominal circumference was significantly ($p<0.05$) reduced in animals belonging to PSHW (Group III, IV, V) compared with HFD (Group II) animals (Fig 2d, e, f).

Organ weight, especially the weight of adipose tissue and liver are important parameter for assessing the diet induced obesity and was found that the weight of liver and adipose tissue reduced significantly ($p<0.05$) with supplementation of different doses of PSHW to animals fed with HFD (Group III, IV, V) compared with HFD (Group II) animals (Fig 2g).

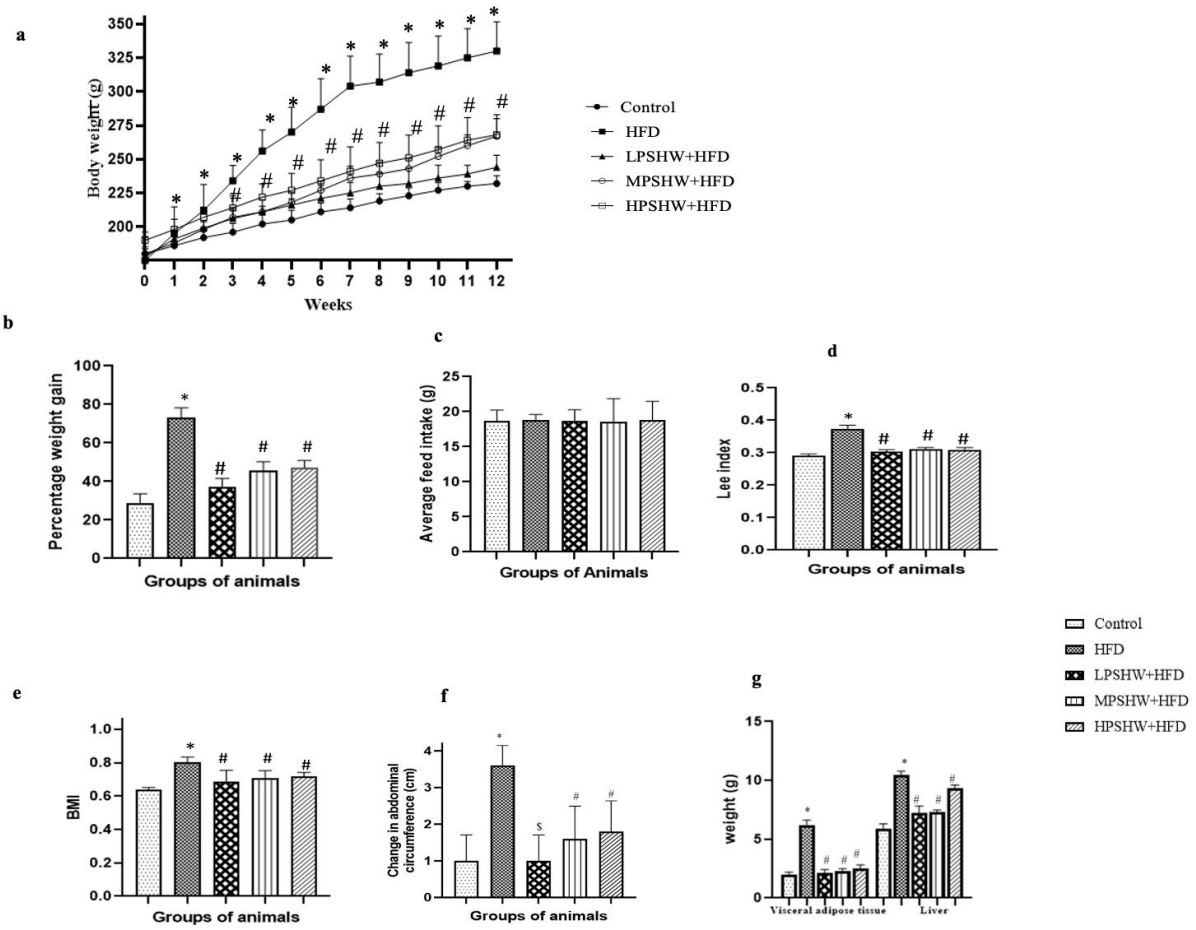

Fig.2Effect of different dose of probiotic supplemented herbal wine on anthropometric parameters: a) Body weight; b) Percent weight gain; c) Average feed intake; d) Lee's index e) BMI; f) Change in abdominal circumference; g) Weight of visceral adipose tissue and liver. Values are Mean±SD, *p<0.01 versus control, #p<0.05 versus HFD, $p<0.01 versus HFD

Fecal Lactic acid bacteria count and fecal lipid

As lactic acid bacteria are the indicator of good gut microbiota therefore it was observed that animals administrated with different doses of PSHW (Group III, IV, V) had significantly (p<0.01) high fecal LAB count and fecal lipid excretion but no significant difference was observed among animals belonging to PSHW (Group III, IV, V) compared with HFD (Group II) animals (Fig 3a, b)

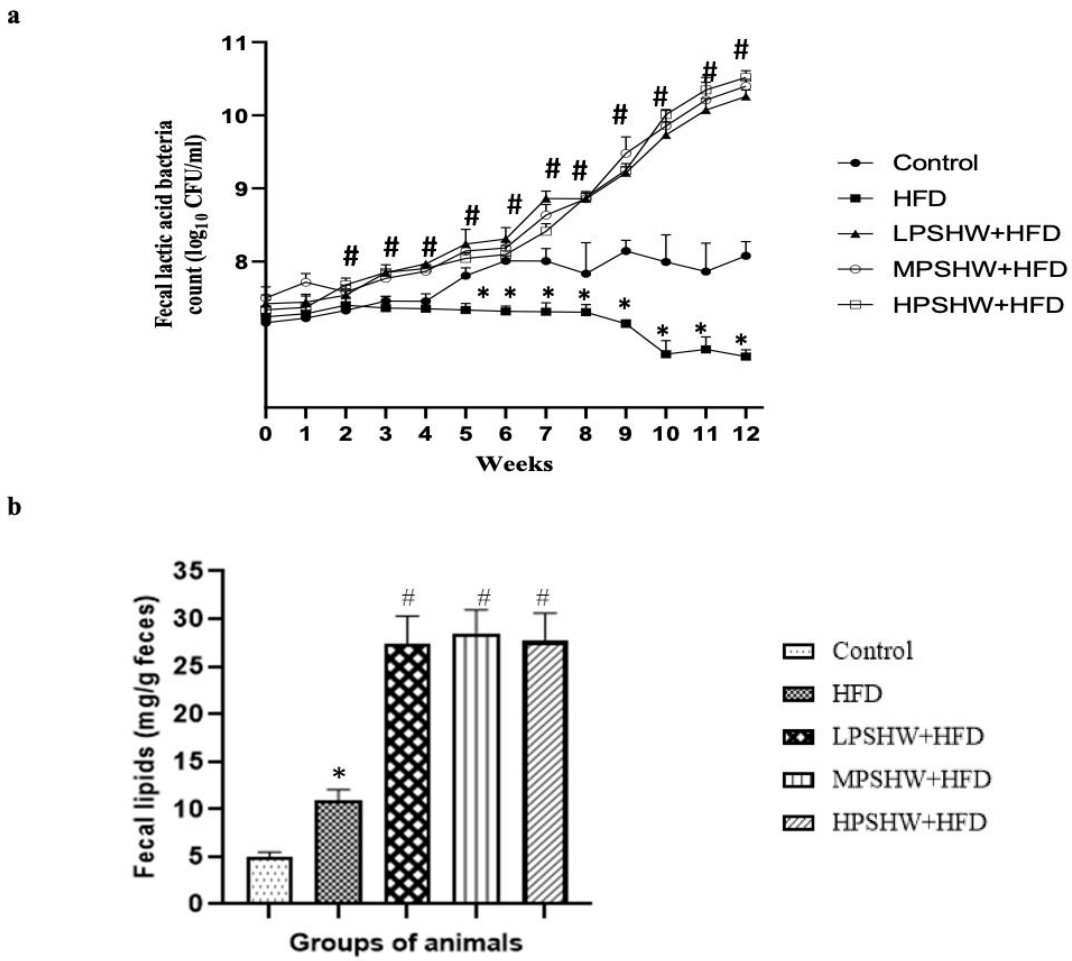

Fig. 3Effect of different dose of probiotic supplemented herbal wine on: a) Fecal lactic acid bacteria (log_{10} CFU/ml); b) Fecal lipid excretion. Values are Mean±SD, *p<0.05 versus control and #p<0.01 versus HFD

Oral glucose tolerance test

Obese individuals are known to have higher fasting glucose level; therefore, oral glucose tolerance test (OGTT) was performed to assess the ability of animals to metabolize glucose after the consumption of high fat diet for 12 weeks. It was found that administration of different doses of PSHW to HFD animals (Group III, IV, V) led to significant ($p<0.05$) reduction in glucose levels compared with HFD animals (Group II; Fig 4a). Further, it was observed OGTT in control animals (Group I) began to clear the excess glucose after 15min of glucose administration while HFD feeding (Group II) led to impaired glucose clearance as excess glucose started clearing after 60min. Interestingly, administration of PSHW to animals fed with HFD (Group III, IV, V) revealed that animals belonging to LPSHW+HFD (Group III) and MPSHW+HFD (Group IV) began to drop the excess glucose after 15min of glucose administration whereas HPSHW+HFD (Group V) began after 30min (Fig 4b). Further, area under the concentration-time curve (AUC) analysis illustrated improved glucose tolerance in both LPSHW+HFD (Group III) and

MPSHW+HFD (Group IV) compared with increased AUC in HFD animals (Group II) showing increased fasting blood glucose level along with impaired glucose clearance from circulation (Fig 4c).

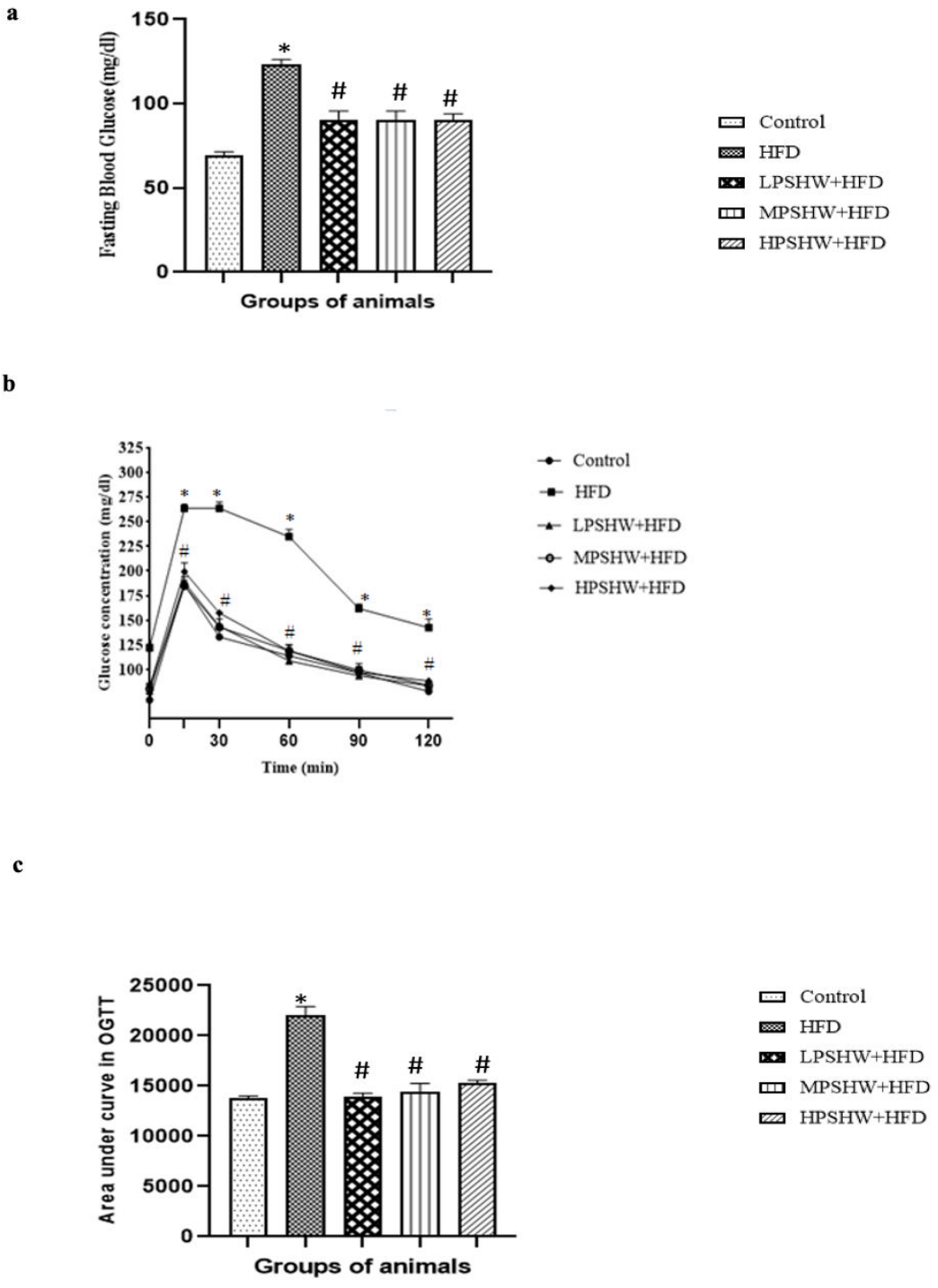

Fig. 4Effect of different of doses of probiotic supplemented herbal wine on: a) Glucose tolerance; b) Fasting blood glucose; c) Area under curve in OGTT. Values are Mean±SD, *p<0.01 versus control and #p<0.05 versus HFD

Serum biochemical parameters

It was observed that administration of different dose of PSHW to HFD animals reduced the level of bilirubin, AST, ALT, total cholesterol and triglyceride significantly (p<0.01) compared with HFD animals (Group II) but maximum reduction was in LPSHW+HFD (Group III) and MPSHW+HFD (Group IV) animals compared with HPSHW+HFD (Group V) animals (Table 1).

Further, it was found that administration of different doses of PSHW to HFD animals increased the level of HDL cholesterol and decreased LDL cholesterol compared with HFD animals (Group II) but significant (p<0.01) improvement was observed in LPSHW+HFD (Group III) and MPSHW+HFD (Group IV) animals compared with HPSHW+HFD (Group V; Table 1).

Table 1: Serum lipid profile and liver function test of animals belonging to different groups.

Parameters	Control	HFD	LPSHW+HFD	MPSHW+HFD	HPSHW+HFD
Bilirubin(mg/dl)	0.53±0.21	1.3*±0.33	0.81$±0.06	0.84$±0.06	0.93±0.04
Aspartate transaminase (AST)(IU/L)	98±9.44	193*±5.02	106$±2.90	108$±6.01	152#±1.1
Alanine transaminase (ALT)(IU/L)	44±10.8	95*±9.6	58$±7.4	62$±13.3	78#±4.18
Total Cholesterol(mg/dl)	84±4.65	109*±3.05	79$±4.09	84$±1.22	93±2.51
HDL cholesterol(mg/dl)	51±4.63	34*±4.06	43±2.56	39±2.66	28±1.87
LDL cholesterol(mg/dl)	36±3.57	68*±5.3	31$±3.49	32$±3.4	39#±2.44
Triglyceride(mg/dl)	46±2.8	122*±3.4	55$±4	51$±6.2	88#±4.5

/alues are expressed as Mean±SD. *p<0.01versus control, $p<0.01 versus HFD and #p<0.05 versus HFD

Histological examination

Histological analysis of liver of control animals (Group I) revealed normal hepatocytes with no deposition of fat and infiltration of inflammatory cells compared with progressive increase in steatosis and inflammatory damage characterized by infiltration of inflammatory cell in HFD (Group II) animals (Fig 5a, b). Interestingly, animals fed with different doses of PSHW showed almost normal hepatocyte and displayed less hepatic steatosis (Fig 5c, d, e)

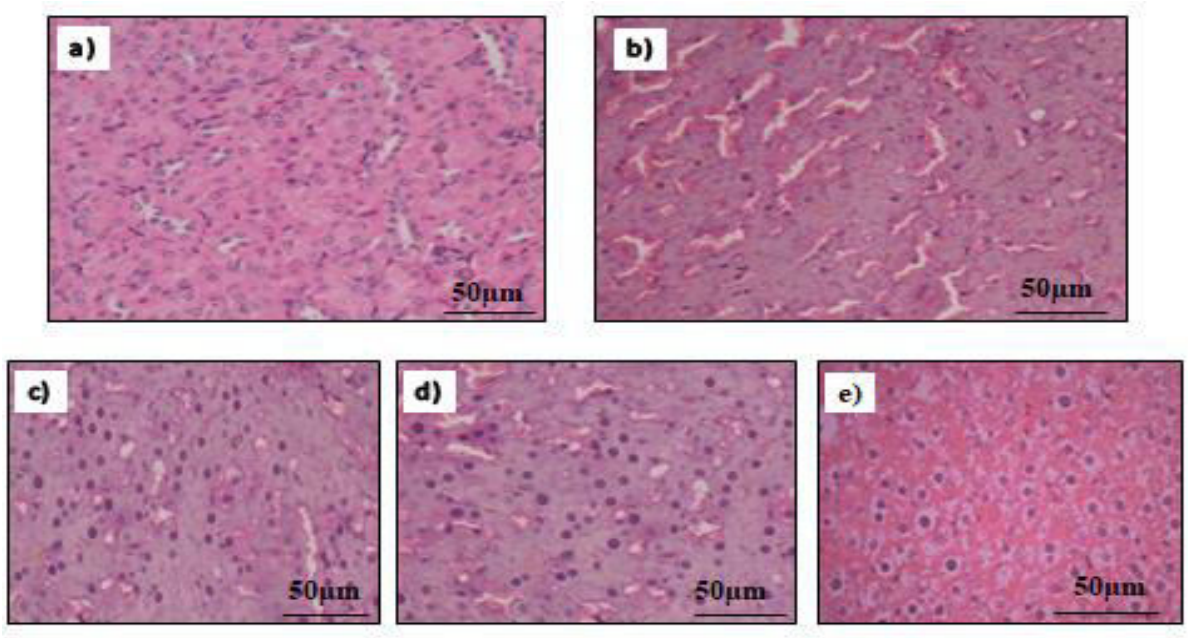

Fig. 5Photomicrograph of liver showing: a) Normal morphology of liver and hepatocytes in control animals; b) Excessive fat deposition in liver indicating hepatic steatosis in HFD animals; c-e) Normal hepatocytes with reduced hepatic steatosis in LPSHW+HFD, MPSHW+HFD, HPSHW+HFD (H&E staining, scale bar: 50µm, 400x; arrow indicates steatosis and normal hepatocytes)

Histopathologically, the colon of control animals (Group I) had closely packed mucus glands; intact epithelium lining, normal microvilli and crypts compared with distorted crypts, microvilli, mucosal epithelial lining and dense infiltration of inflammatory cells in HFD (Group II) animals (Fig 6a, b). More specifically, the prophylactic feeding of different doses of PSHW to animals fed with HFD (Group III, IV, V) attenuated the intestinal damage as is evident by almost preserved architecture of crypts and villi with well-formed mucosal epithelial lining (Fig 6c, d, e).

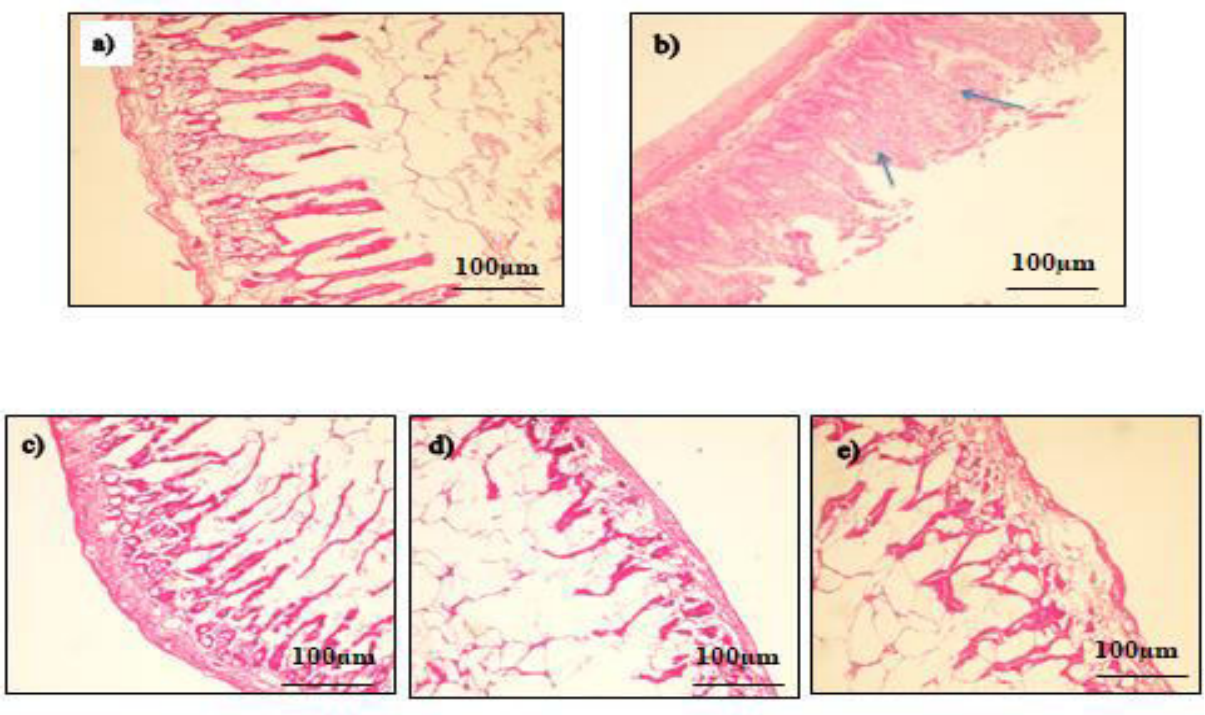

Fig. 6Photomicrograph of colon showing: a) Normal colon morphology with normal crypts in control animals; b) Disrupted crypts with large infiltration of inflammatory cells in HFD animals; c-e) Normal crypts with very less infiltration in LPSHW+HFD, MPSHW+HFD, HPSHW+HFD (H&E staining, scale bar: 100µm, 100x; arrow indicates infiltration of immune cells)

Further, it was found that adipose tissue of control animals (Group I) showed normal morphology compared with excessive lipid accumulation and increased adipose cell size in HFD (Group II) animals (Fig7a, b) while PSHW (Group III, IV, V) animals had reduced adiposity (Fig 7c, d,e).

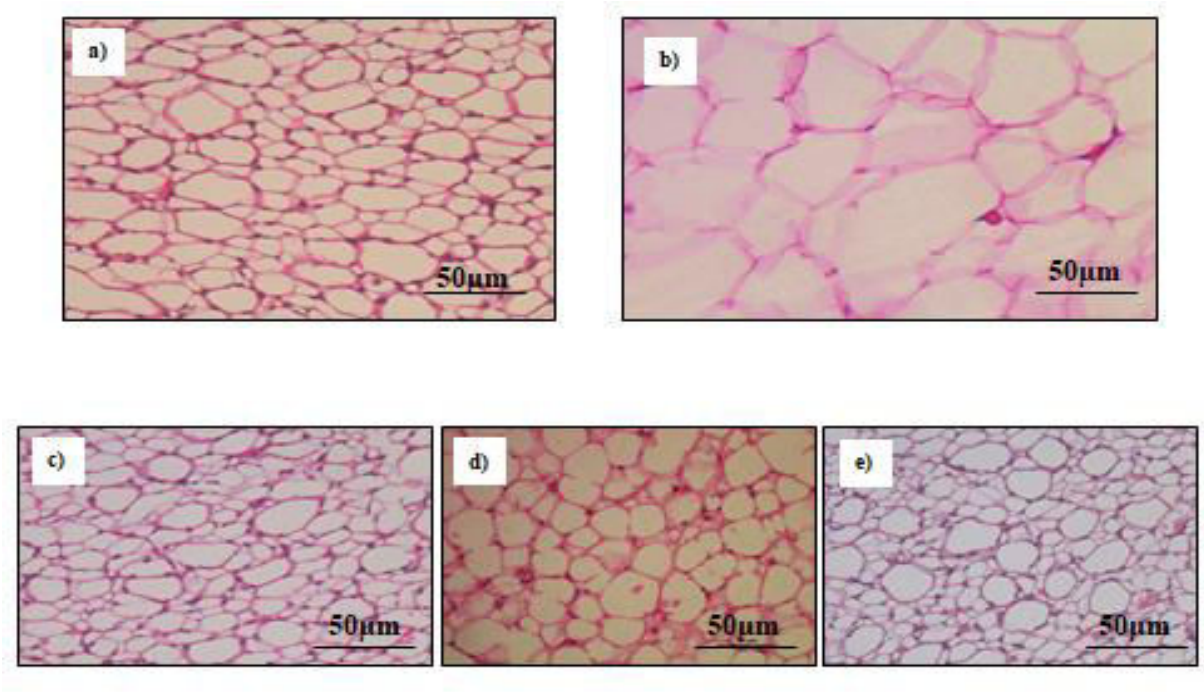

Fig. 7Photomicrograph of adipose tissue showing: a) Normal morphology of adipose tissue in control animals; b) Increased size of adipose cells in HFD animals; c-e) Reduced adiposity in LPSHW+HFD, MPSHW+HFD, HPSHW+HFD (H&E staining, scale bar: 50µm, 400x)

Discussion

The prevalence of metabolic syndrome has expanded to epidemic levels worldwide due to change in life style specifically in relation to diet and physical activity. Surprisingly, it has been seen that some populations consuming even having high fat diet (Greek and French) have low incidence of coronary heart disease referred as "French Paradox" phenomenon mainly attributed due to regular consumption of wine [34]. More specifically, both experimental and clinical investigations of diverse ethnic groups have demonstrated the multiple beneficial effects of moderate wine consumption i.e., attenuating the development of MetS and associated cardiovascular mortalities [35-36]. Therefore, the present study was designed to delineate the prophylactic potential of PSHW in experimental metabolic syndrome.

It was analyzed that the administration of different dose of PSHW for 12 weeks to HFD fed animals led to significant decrease in anthropometric parameters i.e., body weight, Lee's index, BMI, abdominal circumference with no change in average feed intake. This may be due to combinative beneficial potential of probiotic and wine as both are well known to have modulatory potential i.e., anti-inflammatory, immunomodulation along with influence on body weight and abdominalcircumference[37-39]. Recently, Khanna et al. have also demonstrated the prophylactic potential of probiotic *L. pentosus*GSSK2 in experimental metabolic syndrome that modulated the adiposity by increasing fatty acid oxidation, preventing bile salt recycling resulting in inhibition of lipid reabsorption thereby improving the overall functional integrity [15].

It is very well known that consumption of high fat diet enhances the risk of becoming obese vis-à-vis other abnormalities i.e., diabetes, hyperlipidemia an important indicator and component of metabolic syndrome. Interestingly, it was observed that moderate intake of PSHW reduced the fasting blood glucose levels and impaired glucose tolerance compared with HFD animals and correlates with earlier studies [40-41]. These scientists have also observed that moderate wine consumption reduce the risk of diabetes, as wine consumption and diabetes have been linked in a nonlinear way i.e., the risk of diabetes being lowest in light and moderate drinkers and highest in heavy drinkers.

The pathogenesis of diabetes is complex and has been found to be related with oxidative stress vis-a-vis gut microbiome. Interestingly, it was observed that continued administration of moderate PSHW ameliorated the recovery of beneficial LAB in feces indicating restoration of the gut microbiome and ameliorating the gut microenvironment resulting in enhanced insulin sensitivity due to increased production of insulin, adiponectin and decreased inflammation in tissues thereby regulating the glucose metabolism [42-44]. Additionally, the antidiabetic effect of probiotic has been linked to an increase in the levels of short chain fatty acids that may enhance the expression of GLUT-4 transporter leading to increased glucose uptake by skeletal muscles and adipose tissue [45].
Ameliorating potential of PSHW was further monitored by assessing hepatic biochemical markers and it was observed that supplementation of either low and medium dose of PSHW decreased the levels of total cholesterol, triglycerides and increased the level of HDL-cholesterol that is in accordance with earlier studies [46-48]. These scientists have also observed that moderate alcohol consumption enhanced the HDL-cholesterol and decreased total cholesterol and triglycerides. The observed alteration in various biochemical parameters may be due to increased cellular cholesterol efflux and cholesterol esterification. Scientists have also revealed that probiotic supplemented foods (yoghurt) have hepatoprotective potential as they diminish the level of ALT, AST, cholesterol and total bilirubin due to their free radical scavenger property as well as hepatic regeneration [49,50]. The improved biochemical parameters by PSHW are very well supported by restored histoarchitecture of most vital organs (colon, adipose tissue and liver) mainly due to combinative modulatory potential of wine and probiotic as both have anti-inflammatory potential [51, 52].

Based on the observation of present study, the modulatory potential of PSHW in experimental metabolic syndrome may be attributed to remodulation of gut microbiota that may have regulated the energy metabolism causing lipid oxidation and hence preventing the fat accumulation. Additionally, PSHW might have regulated the plasma glucose and insulin resistance by restoring the intestinal permeability vis-à-vis attenuating endotoxemia and leading to reversion in the progression of metabolic complication due to antioxidant potential of PSHW.

Conclusion

In totality, the preliminary observation of the present study, highlights the beneficial potential of PSHW when consumed in moderate quantity in experimental metabolic syndrome w.r.t anthropometric, biochemical and histoarchitecture of organs (liver, adipose tissue, colon). However, further detailed experimental study w.r.t gut microbiome and immuneprofile is underway for the elaborate validation of PSHW as an effective prophylactic agent in experimental metabolic syndrome.

Reference

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Statements and declarations

Competing Interest The authors declare that they have no competing interest.

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Author's contribution Shweta Kamboj performed the experiment and wrote the manuscript; Geeta Shukla gave the idea and edited the paper; Sanjeev Kumar Soni edited the manuscript.

Availability of data and material The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethical approval All animal experiment procedures were conducted in accordance with the guidelines of the Institutional Animals Ethical Committee (IAEC), Chandigarh and approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals **(PU/45/99/CPCSEA/IAEC/2021/536)**