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Metabolic Pathway of Resistant Starch via Micro Biome in Healthy and Hyperglycemic Rats

Salwa B El-Magoli[a], Hanaa H Elsayed[b],Shahira A Al-Gamal[a,c,*], and Mohamed E Abdel-Aziz[a]

[a] *Food science department, Faculty of Agriculture, Cairo Giza 12613, Egypt*

[b] *Nutritional Chemistry and metabolism department, National Nutrition Institute, Cairo, Egypt*

[c] *Food science department, National Nutrition Institute, Cairo, Egypt*

Abstract

Google engines claimed that green banana is a miracle fruit, is this true?

Green banana powder (GBP) was given to diabetic and healthy rats in two different concentrations to see how it affected them. The goal of the planned study was to see how GBP feeding affected diabetic rats. Method: Sixty male albino rats were separated into six groups: the control group was fed a standard diet, while the GV and G VI groups were given 5 and 2.5 percent of a GBP diet, respectively, to assess their safety. To induce diabetes, GII was given an intraperitoneal "IP" injection of streptozotocin (STZ) (40 mg/kg BW). For 30 days, GIII and G IV diabetic rats were given 5 and 2.5 % of the GBP diet, respectively. Rats were exposed to biological evaluation measures throughout the investigation (feed intake, growth curve). Biochemical assays (serum diabetes markers, liver functions; GSH and MDA as antioxidants indication in liver tissue) and histopathological characteristics of the liver and pancreas were performed at the end of the treatment period. Finally, the results showed that the GBP had no physiological deleterious effects in healthy rats throughout the research except for around 20% of the rats, who showed some histopathological alterations after consuming a 5% GBP. From biological and biochemical parameters, treatment with 2.5 % GBP alleviated STZ-induced diabetes more than treatment with 5 % GBP. Conclusion: GBP contains high-resistance starch, which promotes a healthy gut microbiome, and bioactive composites that have a beneficial impact on diabetes via antioxidants and anti-inflammatory effects when consumed in safe doses to avoid negative impacts. Recommendation: Consuming little amounts of green banana for short times may be more useful and safer in terms of diabetes management.

Keywords: Green Banana, Resistant starch, Hyperglycaemia in rats

1. Introduction

Natural plants and functional foods are used worldwide recently to treat diabetes because they are less toxic, low in cost, and free from side effects when compared to synthetic drugs [1]. Functional foods have a beneficial impact on health due to their containing bioactive compounds, which may exist naturally or may be extracted from other sources and added to food; also, it could form during industrial processing. Phytochemicals such as vitamins, peptides, polyphenolic compounds, carotenoids, isoflavones, and resistant starches are some functional food examples. [2]. Banana plants and fruits have enormous health benefits; they have been used as complementary medicine to treat pathological conditions since the ancient past [3]. The green banana powder consists of 70%-80% starch on a dry basis. Starch could be divided into three categories according to its digestive properties: Rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) [4]. Resistant starch (RS) is defined as a fraction of starch that cannot be digested by amylases in the small intestine and is fermented by microbiota in the large intestine producing short-chain fatty acids (SCFA) [5 & 6]. Increased concentrations of SCFA in the large intestine decrease the colonic environment pH, as a result preventing the growth of pathogenic bacterial species [5]. Unripe (green) banana belongs to a resistant starch type 2 (RS2 class) which are starch granules that are shielded from digestion due to their conformation or structure as being crystalline and compact in nature give protection against digestive enzymes and amylases and hence are poorly susceptible to hydrolysis [7]. Green banana is known as a food with a low glycemic index, non-manufactured, appealing, and low-cost food containing different types of fibers, rich in vitamins (B6, biotin, ascorbic acid, and β-carotene), minerals (manganese and potassium), and bioactive compounds with high RS content [8 & 9]. Recently, prebiotics such as RS has been

*Corresponding author e-mail: algamal.shahira@gmail.com.; (Shahira Asem Al-Gamal).
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considered a bio-therapeutic and low-cost strategy for the management of metabolic disorders and the reduction of their adverse outcomes [6]. According to previous studies, it can be concluded that the RS can improve intestinal function, reduce fat accumulation, reduce appetite, and lipid metabolism. Recent investigations have focused on the possible associations between RS and incretions as well as gut microbiota [10 & 8]. A decreasing effect in both postprandial glycaemia and insulinemia may be expected after taking RS, compared with digestible starch. Moreover, though the mechanism of RS is similar to that exerted by dietary fiber, RS may affect the absorption amount of other nutrients in the diet, glucose and fat, which may be useful for controlling glycaemia or lipedemia [11 & 6]. Diabetes mellitus (DM) is a general term for a group of metabolic abnormalities with the dominant feature of chronic hyperglycemia (increased glucose concentrations in the blood). It results from either insufficient insulin secretion or impaired insulin efficiency or, most often, both [12]. In other words, either diabetes is due to the pancreas not producing enough insulin or the body cells not responding properly to the insulin produced [1]. Diabetes mellitus could be divided into two types: insulin-dependent diabetes mellitus (IDDM) or type 1 (insulin-deficient type hyperglycemia) and noninsulin-dependent diabetes mellitus (NIDDM) or type 2 (elevated blood sugar due to inadequate production of insulin or ineffective use of insulin because of insulin resistance). Statistics recorded that more than 90% of diabetic patients are having type 2 diabetes [1]. DM is one of the main global health challenges [13] as it is a critical and complex chronic condition, which is the main cause of defected health all over the world. Diabetes-related deaths are increasing considerably despite the new treatment and drugs utilizable [14]. Worldwide predictions suggest that more than 300 million people will have diabetes by the year 2025 [15]. According to the International Diabetes Federation (IDF), the number of world adults with diabetes in 2019 was 643 million and it was suggested that this number will increase to 700 million by 2045 [14]. Streptozotocin (STZ) has been used in the field of research for many years to cause diabetes mellitus permanently in rats and mice due to its specific toxic effect on pancreatic β-cells [16]. It is synthesized by a gram-positive bacterium strain of the soil microbe *Streptomyces achromogenes*, which has a broad spectrum of antibacterial properties. STZ is transported via low-affinity glucose transporter- GLUT2 of β-cells into the cell and causes alkylation of DNA and irreversible necrosis of β cells. DNA synthesis in mammalian and bacterial cells is prohibited by the action of STZ [16]. STZ is widely used to induce both (Type 1 DM) and (Type 2 DM).

Therefore, the intended study's purpose was to see how GBP feeding influenced diabetic rats with differing concentrations.

2.Materials and Methods:

Streptozotocin was obtained from Sigma Chemicals Co. All kits of the biochemical parameters were purchased from Morgan Chemical Company, Egypt. •Commercial hard fresh Ecuadorian Green banana fruits are obtained from the local market containing 19.2 % resistant starch (RS) according to the exported product SPECIFICATION SHEET 2018.

•Rats: 60 male Sprague–Dawley rats (150g ± 20) were obtained from the animal colony, Helwan Farm, Vaccine and Immunity Organization, Helwan Governorate, Egypt.

•Diets: The experimental diets were prepared according to the methods for Reeves, *et al.,* [17].

2.1. Green banana powder preparation and chemical evaluation:

Fruits were peeled and cut into 1cm slices, immediately rinsed in citric acid solution (0.3% w/v). The slices were dried at 50ºC, ground using a commercial grinder, and stored at 25ºC in sealed plastic containers for further analyses [18].

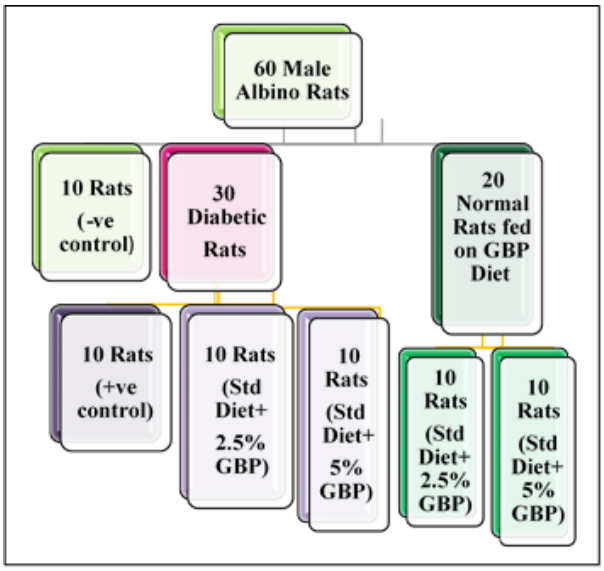
2.2. Chemical analyses:

Analysis of moisture, ash, protein and fat were performed according to A.O.AC [19].

- Moisture % was determined using an air draft oven for most food items.
- Protein % was calculated from the total nitrogen values (determined by Kjeldahl method).
- Fat % was determined by Werner Schmidt method, which involves acid hydrolysis followed by extraction of fat with organic solvent.
- Ash% was determined using muffle furnace at 550 C.
- Total Carbohydrate % by difference. Carbohydrates (g) = 100- (moisture + protein + fat + ash + crude fiber).
- Crude fiber % was determined as residue after acid digestion followed by alkali digestion according to the method of Maynard [20].
- Dietary fiber % was estimated in compliance with the method of AOAC [21].

2.3. Induction of diabetes:

Diabetes was induced in rats by intraperitoneal injection of STZ diluted in 0.5 ml of 0.1 M sodium citrate buffer pH 4.5 at a dose of 40 mg/kg body weight after an overnight fast (no food for 12 hours, but free access to water). They have unrestricted access to food and water following the injection. To overcome hypoglycemia, the animals were given a 5 % glucose solution to drink overnight [16]. After 72 hours after STZ injection, the onset of diabetes was established. Diabetic rats were defined as animals with fasting blood glucose levels of greater than 250 mg/dl and were employed in experiments [22].



2.4. Experimental design:

2.4.1. Biological experiment:

After 5 days, the rats were divided into three main groups as follows:

Experimental rats (60 healthy adult male rats) were housed in well aerated wire cages as individual under hygienic conditions, standard lighting (12-h daylight and 12-h dim period) and the room preserved at 25±2 °C throughout the experiment. Food and water were provided and checked daily. Rats fed on a standard diet for 5 days as adaptation before experimentation and approved from the National Hepatology & Tropical Medicine Research Institute (SN. A3-2022).

2.4.2. Biological evaluation:

Animals and diet were weighed twice a week. At the end of the experiment body weight (g) and feed intake (g) were calculated as a mean and standard error for each group.

2.4.3. Biochemical analysis:

After one month of experimentation, the rats were fasted overnight and then sacrificed after slight anesthesia, and blood was taken from the venous sinus through rat eyes using fine capillary glass tubes. The plasma was collected in EDTA tubes (stored in the fridge), serum and fluoride tubes (stored at -20^{o} C) for tests. Estimation of diabetes biomarkers and liver functions in serum, also MDA and GSH in liver tissue were determined according to biochemical methods described below then the mean values were calculated.

I. Diabetes biomarkers:

- Fasting blood glucose (FBG) was determined according to Barhamand Trinder[23].
- Fasting insulin (FI) was estimated in accordance with Rudovich, *et al*., [24].
- Homeostatic model assessment of insulin resistance: (HOMA-IR) index; calculated as follows in agreement with Diab, *et al*., [25].

HOMA-IR = [Fasting serum insulin (µIU / mL) × Fasting serum glucose (mmol / l)] / 22.5

- Homeostatic model assessment of pancreas beta-cell: HOMA-β is calculated as follows in line with the equation of Park, *et al*., [26].

HOMA-β = [360 × Fasting serum insulin (µIU/ mL)] / [Fasting serum glucose (mg /dl) – 63].

- Insulin sensitivity index (ISI-QUICKI) = 1/ (Log Io + Log Go)
  - I: Fasting insulin in µU/ml. - G: Fasting blood glucose in mg/dl.
- HbA1c was determined in compliance with the method of Tietz[27].
- LDH was assessed according to the method of Van, *et al*., [28].

II. Liver functions:

- Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to the method of Huang, *et al*.,[29].
- Alkaline phosphatase (ALP) was measured according to Friedman [30].
- Total Protein (TP) was analyzed according to Kaplanand Szalbo [31].

III. Lipid peroxidation as Malondialdehyde and Glutathione in the liver:

- Malondialdehyde (MDA) could be measured calorimetrically according to the method of Miharaand Uchiyama [32].
- Glutathione (GSH) was determined according to Beutler,*et al*., [33].

2.5. Histopathological Examination of Liver and Pancreas: It was carried out according to Lambergton and Rothstei [34].

The liver and pancreas of every rat were removed by careful dissection, washed in saline (0.9%), dried by filter

paper then weighed, and part of the liver and the whole pancreas were placed in 10% formalin for histopathological examination.

2.6. Statistical analysis:

Data are expressed as Mean ± SD and were analyzed by one-way analysis of variance (ANOVA) followed by test significant difference (LSD) multiple comparison tests (SPSS version 28); P-values < 0.05 are considered statistically significant [35].

3. Results and discussion:

3.1. Chemical analysis of green banana powder:

Table 1: Chemical composition of GBP:

Parameters	Result by (%) in GBP
Moisture	**10**
Ash	**3.4**
Protein	**4.8**
Fat	**0.15**
Total carbohydrates	**81.65**
Crude fiber	**1.3**
Dietary fiber	**4.2**
Calories	**347.2**

3.2. Biological evaluation:

Feed intake (g/day) and Growth curve (BW. g/week) for each group:

Figures (1) and (2) illustrated that there were significant differences in feed intake (FI) and body weight gain (BWG) within all groups. Feed intake (FI) in the diabetic rats (G2) as a positive control group was significantly higher than all other groups, associated with a significant decrease in body weight (BW) when compared to other groups in the study. This is normal according to Sureshlal [36] because diabetes is associated with intense hunger and unusual weight loss, as the blood insulin is not working properly, or is insufficient that pushes body cells (these cells aren't getting glucose) to seek more energy through eating more food. In addition to breaking down muscle and fat tissues for energy, leading to weight loss.

Diabetic G3 supplemented with (5% GBP) showed increased FI, which then began to normalize from day 21 associated with a significant decrease in BW when compared to all other groups under study. This is due to the same reason discussed for diabetic G2, and also, (5% GBP) contains more available starch than (2.5% GBP), which may be the reason behind increasing blood glucose levels a little as it is digested easily and to some extent may hinder the effect of RS in regulating hyperglycemia efficiently.

A systematic review by Zaky, *et al*., [37] demonstrated by several studies that pathogenic bacteria are found in large amounts in diabetic rats, while beneficial bacteria are lowered compared to control, also a decreased microbial diversity and increased gut permeability were found in the DM type 1 group compared to other groups under study. Additionally, those with DM type 2 had decreased levels of Butyrate-producing bacteria such as *Bifidobacterium*, reduced levels of the *Firmicutes* phylum, and increased levels of bacteria involved in chronic inflammation as *Dorea*. Intestinal microbiota composition did not change after STZ according to the findings of Yin, *et al*., [38].

In the case of diabetic G3, the current study may conclude that (5 % GBP) offered more available starch than (2.5 % GBP), which was easily digested and converted to glucose that was consumed by pathogenic bacteria and this aided in increasing their population over beneficial ones. Due to the reduced number of beneficial bacteria, a portion of the prebiotic RS was fermented and transformed into SCFAs.

Moreover, diabetic G4 (2.5% GBP) had lower FI and higher BW than diabetic G2 and G3. Also noticed that healthy G5 (5% GBP) and G6 (2.5% GBP) had lower FI when compared to normal control G1 from day 15. This may be due to resistant starch (RS) content in GBP, which escapes digestion and is fermented by large intestine microbiota into SCFAs that are acetate, propionate, and butyrate with an approximate molar ratio of 60:20:20, respectively according to Pascale, *et al*., [39]. Additionally, 95% of those SCFAs are absorbed via the intestinal mucosa, while 5% are discharged in the feces according to the findings of Parada, *et al*., [40], where, acetate can cross the blood-brain barrier leading to a reduction in appetite and increases the feeling of fullness [41]. The other two primary SCFAs propionate and butyrate, even in small concentrations affect the brain and hormones as well [42], where they reduced appetite [43]. SCFAs reaches the systemic circulation, and other tissues, regulating whole-body energy homeostasis, which is a homeostatic regulation of food intake (energy inflow) and energy expenditure (energy outflow) [42]. SCFAs (especially butyrate) were proven by Huang, *et al*., [44] that they restored the conformation of gut microbiota, maintained the integrity of the gut epithelial barrier, and have been displayed to modulate intestinal hormones (such as glucagon-like peptide 1 and peptide tyrosine-tyrosine). Affect intestinal permeability, satiety, gastric emptying, and food intake [44], where these circulating hormones are considered satiety signals, as they have been shown to reduce food intake, body weight, and obesity in rodents [45].

Additionally, may conclude that healthy groups G5 and G6 had normal balanced gut micro-biome, which aided in benefiting fully from all RS content provided, and in the case of diabetic G4 supplemented with (2.5% GBP), beneficial bacteria were abundant over pathogenic ones.

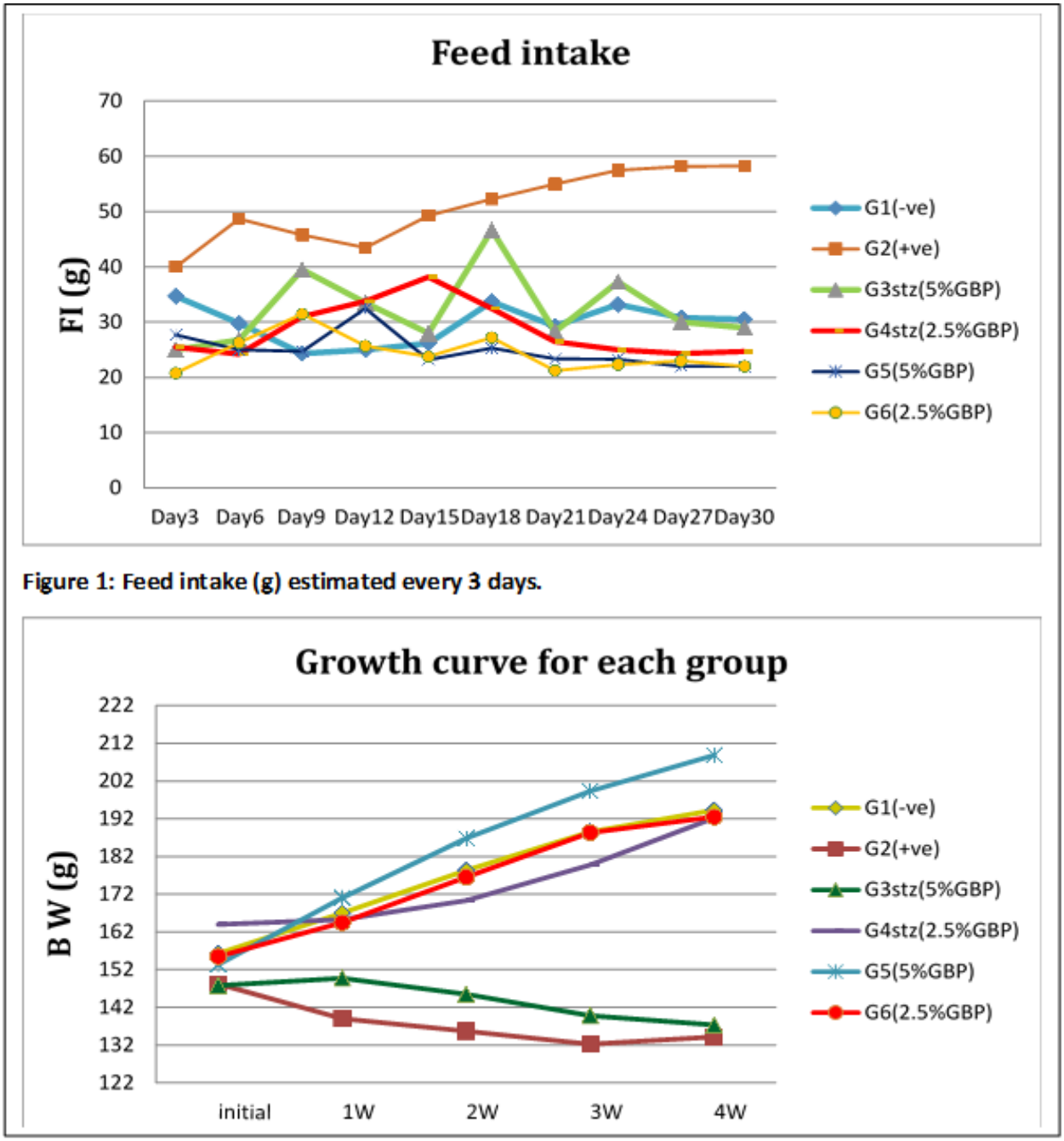


Figure 1: Feed intake (g) estimated every 3 days.

3.3. Biochemical analysis:

I. Diabetes markers:

Statistical results in table (2) revealed that the diabetic group G2 had elevated fasting blood glucose (FBG) value with significant differences when compared to normal group G1. These results were in harmony with the findings observed by Goud, *et al*., [16] and Suman, *et al*., [46] who concluded that STZ at 40mg/kg BW induced diabetes and marked higher glucose levels in the diabetic rats than in normal ones. It has been also reported that GLUT4 (carrier protein) is reduced in type2 diabetes [47], where, at the cell surface, GLUT4 which is insulin-responsive glucose transporter, allows the diffusion of circulating glucose down its concentration gradient into muscle tissues and fat cells [48].

On the other hand, treatment of diabetic groups with a 5 % (G3) and 2.5% (G4) GBP supplemented diet, revealed a significant reduction in FBG when compared to G2, but it did not reach normal control level. Healthy rat groups, G5, and G6 exhibited more decrease in FBG in comparison with G1. Where, (5% GBP) gave better FBG than (2.5 % GBP) in healthy rats.

Table 2: Diabetic biomarkers in groups understudy:

Parameters	G1	G2	G3	G4	G5	G6	*P*-value
FBG (mg/dl)	**89±4.2**	**465.2±7.2**	**275±8.3**	**183.4±3.6**	**81.3±1.7**	**84±4.9**	**0.0000**
% change vs normal control	0	422.7	209	106.1	-8.7	-5.6	
% change vs positive control	-80.9	0	-40.9	-60.6	-82.5	-82	
Insulin (mIU/l)	**1.7±0.5**	**0.71±0.1**	**1.25±0.4**	**1.35±0.4**	**1.52±0.3**	**1.53±0.3**	**0.6306**
% change vs normal control	0	-58.2	-26.5	-20.6	-10.6	-10	
% change vs positive control	139.4	0	76	90.1	114	115.5	
HOMA IR	**0.32±0.084**	**0.82±0.044**	**0.65±0.16**	**0.56±0.18**	**0.28±0.064**	**0.3±0.056**	**0.0128**
% change vs normal control	0	156.3	103	75	-12.5	-6.25	
% change vs positive control	-61	0	-20.7	-31.7	-66	-63.4	
HOMA β	**23.5±2.64**	**0.72±0.11**	**2.1±0.63**	**3.93±1.2**	**29.52±4.3**	**26.43±10.9**	**0.0006**
% change vs normal control	0	-96.9	-91.1	-83.3	25.62	12.5	
% change vs positive control	3163.9	0	191.7	445.8	4000	3570.8	
Insulin sensitivity index	**0.52±0.029**	**0.37±0.004**	**0.41±0.004**	**0.43±0.006**	**0.55±0.009**	**0.53±0.017**	**0.0000**
% change vs normal control	0	-28.8	-21.2	-17.3	5.8	1.9	
% change vs positive control	40.5	0	10.8	16.2	48.6	43.2	
HbA1c (%)	**3.75±1.2**	**11.42±0.4**	**8.53±1.1**	**7.13±1.3**	**3.18±0.35**	**3.42±0.37**	**0.0000**
% change vs normal control	0	204.5	127.5	90.1	-15.2	-8.8	
% change vs positive control	-67.2	0	-25.3	-37.6	-72.2	-70.1	

G1= normal control *G2= diabetic rats positive control* *G3 = diabetic rats treatment with 5% GB*

G4= diabetic rats treatment with 2.5% GB *G5 =treatment with 5% GB* *G6 =treatment with 2.5% GB*

P-values < 0.05 are considered statistically significant

These results possibly contributed to the glucose regulating effect of the SCFAs. A study by Hernández, *et al*., [49] demonstrated that acetate affects the liver leading to an increase in insulin sensitivity, GLUT4, and increased activity of the Adenosine monophosphate-activated protein kinase (AMPK), where activation of AMPK improved insulin sensitivity and glucose homeostasis according to Gupta, *et al*., [50]. Activated AMPK provokes glucose uptake and fatty acid oxidation as energy-generating processes and reduces energy-consuming processes such as protein and lipid synthesis. Mostly, AMPK activation improves insulin sensitivity and glucose equilibrium, which is the balance of insulin and glucagon to maintain blood glucose levels [50]. In the absence of insulin or exercise, 90% of GLUT4 remains intracellular [51].

Moreover, Liu, et al., [43] reported to enhance blood glucose levels, stated acetate. Propionic acid improved glucose tolerance and insulin sensitivity as was observed by [52]. In addition, it had been proved that administrating acetate and propionate orally decreased hyperglycemia in diabetic mice in line with Den, *et al*., [53].

It was concluded by Deng, *et al*., [54] that SCFAs can increase the expression of GLUT4 and translocate it to the cell layer membrane, promoting the absorption of more glucose by myoblasts (an undifferentiated cell capable of giving rise to muscle cells).

Fasting blood insulin (FBI) value in G2, expressed lower insulin levels than G1 with significant differences. This happened due to the toxic effect of STZ targeting pancreatic beta cells causing damage, which leads to impaired production or mark-able decrease in insulin levels circulating through the body [55]. On the other hand, diabetic groups G3 and G4 showed a significant increase in fasting insulin when compared to G2. This could be attributed to the effect of both SCFAs acetate and propionate in rescuing pancreatic beta cells from death and protecting them from the damages as was studied by Liu, *et al*., [43], which led to restoring the functionality of some pancreatic beta cells and in return a normal and sufficient amount of insulin was produced. Generally, (2.5% GBP) gave better FBI than those fed on (5% GBP) in diabetic rats. FBI in healthy rat groups G5 (5% GBP), and G6 (2.5% GBP), were below that of G1. This may be due to modulation of blood glucose by SCFAs produced by GBP, increased insulin sensitivity, increased AMPK activity, and increased whole-body energy homeostasis in those groups as was studied by

Hernández, *et al*., [49] leading to a slight decrease in needing to secret insulin than normal G1.

Homeostatic model assessment of insulin resistance (HOMA-IR) reading value of ≥ 2.7 is considered an indicator of insulin resistance (IR) according to Diab, *et al*., [25]. Statistical results in table (2) showed no IR among groups under investigation as all HOMA-IR values calculated were below 2.7, but it was noticed that G2 has a significantly higher value in comparison to G1. Moreover, (2.5% GBP) showed more decrease in HOMA-IR than (5% GBP) in diabetic rats and vice versa happened in healthy groups. All groups under study manifested a decrease in HOMA-IR when compared to G2. Homeostatic model assessment of pancreas beta cell (HOMA-β) values in table (2) revealed that G2 has a significantly lower value in comparison to G1. Diabetic rats fed on (2.5% GBP) gave a significant increase in (HOMA-β) values than those fed on (5% GBP). In addition, (5% GBP) gave better results in healthy rats.

Statistical results in table (2) demonstrated that the diabetic group G2 presented a significant increase in HbA1c (%) compared to G1. Moreover, lower HbA1c (%) was achieved by (2.5%GBP) in case of diabetic groups and by (5%GBP) in case of healthy rats.

II. Liver functions:

Liver abnormalities such as fibrosis, abnormal fat, and glycogen deposition, cirrhosis, and increased hepatic enzyme activities are associated with diabetes [56].

As presented in table (3) statistical results point out that there was a significant rise in ALT, AST and ALP in G2 versus other groups under study. This significant elevation is due to hepatocellular damage, which led to leakage of these marker enzymes from the cytosol of hepatocytes into the bloodstream, and this result is consistent with the findings reported by Ghanbari, *et al*., [57]. Where hyperglycemia could lead to the storage of too much glucose as glycogen, and fat could accumulate within liver cells, which is toxic. This accumulation lead to damages in organs and tissues throughout the body, particularly the liver and kidneys, resulting in elevated liver enzymes according to the findings of Julián, *et al*., [58].

Generally, (2.5% GBP) showed more decrease in ALT, AST and ALP than (5% GBP) in diabetic groups and vice versa in case of healthy ones.

Table (3) also presented that total protein (TP) for G2 revealed a significant decrease when compared to G1.

Therefore, decreased amount of total proteins in the untreated diabetic rats is an indication of the diminished synthetic function of the liver as was discussed by Sunmonu, *et al*., [59]. Moreover, (2.5% GBP) gave higher TP than (5% GBP) in diabetic rats and vice versa in healthy ones. Generally, diabetic groups G3 and G4 fed on GBP showed a significant decrease in ALT, AST, and ALP associated with a significant increase in TP than G2. These results may be due to SCFAs exerting anti-inflammatory, anti-oxidant, and hypoglycemic effects [44], which aided in liver healing and caused regulation of liver mitochondrial function [42]. Besides, SCFAs are water-soluble which is the reason behind being rapidly absorbed in the large intestine and transported to the liver via the hepatic portal bloodstream. Where they are readily metabolized instead of being stored as fat [60], that in return regulated liver enzymes and increased TP production, moreover, acetate lowers metabolism of fat (decrease fat storage) and lowers the effect of insulin on fat accumulation as was studied by Yamashita, *et al*., [61] and Liu, *et al*., [43]. SCFAs have a positive effect on non-alcoholic fatty liver disease (NAFLD) by regulating the inflammatory response, fat metabolism, and Liu, et al., [62], discussed glucose metabolism in liver tissue after entering the liver through the portal vein as. In addition, Liu, *et al*., [62] concluded that butyrate could enhance the gut barrier and delay the occurrence of Type 1 DM, and NAFLD.

Additionally, statistical results presented in table (3) showed that G2 revealed a significant increase in lactate dehydrogenase (LDH) when compared to G1 and this result was in agreement with that of Ahmed, *et al*., [63] and Farswan, *et al*., [64]. High LDH is due to the hyperglycemic damaging effect on the liver [56] and on gut, micro-biome [37] and intestinal barrier [65], where it induced mitochondrial dysfunction and enhanced oxidative stress [66]. Also caused the deterioration of the intestinal barrier and stimulation of liver damage through the gut– liver axis [67]. Gut barrier damage results in the leakage of microbes, microbial metabolites, and products from the gut lumen to the portal vein and systemic circulation [68]. While (2.5% GBP) gave lower LDH values than (5% GBP) in diabetic rats and vice versa in healthy ones. This may be attributed to the effect of SCFAs, which reversed diabetic hepatocellular damage, destruction of the intestinal barrier and gut micro-biome according to the findings of Liu, *et al*., [62]. Where SCFAs maintained the gut barrier by promoting the repair of intestinal epithelial cells, restoring the intestinal tight connection, and regulating the activities of gut microbiota that aided in liver recovery, also GBP and SCFAs had an anti-inflammatory and anti-oxidant effect, which affected liver positively by reducing oxidative stress and overall inflammation [42].

Table 3: Liver functions and LDH:

Parameters	G1	G2	G3	G4	G5	G6	*P*- value
ALT (U/L)	**45.6±5.8**	**133.3±5.7**	**57.1±7.4**	**50.7±10.5**	**35.2±2.9**	**49±7.6**	**0.0000**
% change vs normal control	0	192.3	25.2	11.2	-22.8	7.5	
% change vs positive control	-65.8	0	-57.2	-62	-73.5	-63.2	
AST (U/L)	**141±11.6**	**339.8±28.3**	**241±11.2**	**225±9.1**	**143.2±14.1**	**144.75±7.3**	**0.0000**
% change vs normal control	0	141	71	59.6	1.56	2.7	
% change vs positive control	-58.5	0	-29	-33.8	-57.9	-57.4	
ALP (U/L)	**406.7±13.1**	**2024.2±37.8**	**643.8±71.3**	**531.6±40.2**	**213±14.4**	**258±12.7**	**0.0000**
% change vs normal control	0	397.7	58.3	30.7	-47.6	-36.5	
% change vs positive control	-79.9	0	-68.2	-73.7	-89.5	-87.2	
Total protein(g/dl)	**7.4±0.4**	**4.1±0.2**	**5.9±0.5**	**6.1±0.6**	**7.7±0.17**	**7.5±0.23**	**0.0000**
% change vs normal control	0	-44.6	-20.3	-17.6	4.1	1.4	
% change vs positive control	80.5	0	43.9	48.8	87.8	83	
LDH (U/L)	**4234.2±290.1**	**11924±522.8**	**8074.2±319.4**	**6745.2±210**	**3937.2±420**	**4036.2±290**	**0.0000**
% change vs normal control	0	181.6	90.7	59.3	-7	-4.7	
% change vs positive control	-64.5	0	-32.3	-43.4	-67	-66.2	

G1= normal control *G2= diabetic rats positive control* *G3 = diabetic rats treatment with 5% GB*

G4= diabetic rats treatment with 2.5% GB *G5 =treatment with 5% GB* *G6 =treatment with 2.5% GB*

P-values < 0.05 are considered statistically significant

Table 4: GSH and MDA in the liver of rats under investigation:

Parameters	G1	G2	G3	G4	G5	G6	*P*-value
GSH Liver (m.mol/g liver tissue)	**45.3±7.8**	**13.4±2.14**	**32±4.3**	**40.4±0.43**	**50.8±3.2**	**47.6±4.8**	**0.0000**
% change vs normal control	0	-70.4	-29.4	-10.8	12	5	
% change vs positive control	238	0	138.8	201.5	279	255.2	
MDA Liver (m.mol/g liver tissue)	**15.3±1.2**	**29.8±0.65**	**21.9±1.84**	**16.7±2**	**12.3±2.89**	**14.08±1.22**	**0.0000**
% change vs normal control	0	94.8	43	9.2	-19.6	-8	
% change vs positive control	-48.7	0	-26.5	-44	-58.7	-52.7	

G1= normal control *G2= diabetic rats positive control* *G3 = diabetic rats' treatment with 5% GB*

G4= diabetic rats treatment with 2.5% GB *G5 =treatment with 5% GB* *G6 =treatment with 2.5% GB*

P-values < 0.05 are considered statistically significant

III. Glutathione (GSH) and Lipid Peroxidation by Malondialdehyde (MDA) in the liver tissue:

Statistical results in table (4) demonstrated that GSH in liver tissues of G2 is significantly lower than G1. While MDA in liver tissues of G2 is significantly higher than G1. Generally, G2 showed significantly lower GSH values and higher MDA values in the liver than in other groups. Fluctuations in oxidative stress levels in diabetic animals are due to autoxidation of glucose, protein glycation, lipid peroxidation, and low antioxidant enzyme activities [69]. Inconsistent with these findings, the present study revealed that increased MDA levels and decreased GSH levels were seen in the liver of the STZ-

induced diabetic rats and this is in agreement with the study of Sheweita, *et al*., [70]. In addition, it is in agreement with another previous study [71], which showed that GSH levels were decreased during different diabetic stages.

Generally, diabetic rats fed on (2.5%GBP) gave higher liver GSH and lower MDA levels than (5% GBP). While, (5% GBP) was better in case of healthy rats.

This outcome may be attributed to more anti-oxidant, polyphenols, flavonoids, RS and anti-inflammatory contents [72] present in (5% GBP) diet in case of healthy G5 and more utilization of RS and other nutritional contents in (2.5% GBP) by micro-biota in case of diabetic G4. Where SCFAs produced (due to GBP fermentation) exerted an anti-inflammatory and anti-oxidant effect according to the findings of González, *et al*., [60].

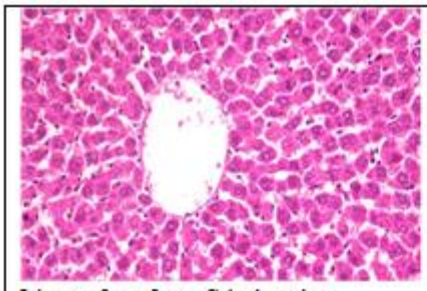
4.Evaluation of histopathological changes in the liver and pancreas:

Microscopically as was shown in figure (8), the liver of rats from G1 revealed normal histological structure of hepatic lobule, on contrary, the liver of rats from G2 described hepatocellular necrosis combined with inflammatory cells infiltration, fibroplasia in the portal triad, and portal infiltration with inflammatory cells. The reason behind these changes is STZ-induced diabetes which is characterized by abnormal fat and glycogen deposition and increased production of reactive oxygen species (ROS) which are involved in hepatic damage by causing necrosis, inflammation, and oxidative stress in hepatic tissues [73]. Meanwhile, some liver sections of rats from G3 described slight vacuolar degeneration of some hepatocytes and slight Kupffer cells activation. This could be attributed to diabetes but we may conclude that SCFAs from (5% GBP) showed some protective effect against the progression of diabetic complications in the liver. Kupffer cells are the main phagocytic or immune cells of the liver [74]. Some substances may damage or lower the activity of Kupffer cells, other agents may enhance Kupffer cell activity. Kupffer cells can have a role in the pathogenesis of toxic hepatic damage and repair in diverse ways [74]. On the other hand, the liver of rats from G4 revealed marked Kupffer cells activation, which is attributed to SCFAs effect and hepatocellular steatosis because of the accumulation of excessive glycogen and fat in the diabetic liver. Activated Kupffer cells due to hepatic toxicity may also participate in the repair of injured hepatic parenchyma by generating new hepatocytesand reversing fibrosis [74]. Some examined sections from G4 rats revealed no histopathological alterations. Additionally, the liver of rats from G5 and G6 showed no histopathological alterations as was shown in fig (8), while some rats from G5 revealed some histopathological changes as was shown in figure (10).

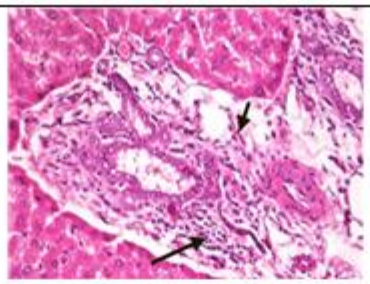
Microscopically examination of the pancreas of rats from G1 revealed normal pancreatic acini and normal islets of Langerhan's as was shown in figure (9), on contrary, the pancreas of rats from G2 exhibited marked vacillations of cells of islets of Langerhan's and vacuolation of cells lining pancreatic acini due to STZ that transferred via GLUT2 transporter into the β-cells and its accumulation caused β-cell death as a result of DNA alkylation [73]. However, pancreas of G3 rat showed vacuolation of cells of islets of Langerhan's and vacuolation of cells lining pancreatic acini. Meanwhile, the improved picture was recorded in sections from G4, examined pancreas revealed vacuolation of some cells of islets of Langerhan's, and some examined sections from G4 exhibited no histopathological alterations, which could be imputed to the protective effect of SCFAs obtained from (2.5% GBP). On the other hand, the pancreas of rats from G5 and G6 exhibited no histopathological alterations except for some rats from G5 as was shown in figure (10).

Figure (10) revealed histopathological alterations in the liver and pancreas, which may be attributed to increased concentration of butyric acid as was concluded in an investigation done by Most, *et al*., [75], which found that the liver of mice could tolerate lower butyric acid concentrations, on the other hand, increased concentrations lead to degenerative hepatocellular changes. In addition, Most, *et al*., [75] discussed that diets containing butyric acid as an SCFA could facilitate weight gain and cause obesity in various animal models and stated that treatment with butyric acid reduced the total serum cholesterol levels, but high butyric acid doses increased cholesterol levels in mice. It was considered by Most, *et al*., [75] that this could lead to the development of NAFLD (fat accumulation in the hepatocytes). Lipid accumulation in large amounts can develop into non-alcoholic steatohepatitis then cirrhosis, and, finally, hepatocellular carcinoma. A study by KoyuncuSokmen, *et al*., [76] discussed that pancreas and liver tissues arise from the same endoderm embryologically, which could lead to ectopic fat accumulation in both organs, additionally, their literature review showed that pancreatic tissue is highly sensitive to fat accumulations than liver tissue. Fatty liver develops due to intracellular lipid accumulation in hepatocytes, while fat accumulation in the pancreatic tissues occurs due to intercellular adipocyte (fat cells) infiltration in both acinar and islet cells in the interlobular region. Pancreatic steatosis begins with fat accumulation in the pancreas to pancreatic inflammation then development of pancreatic fibrosis [76].

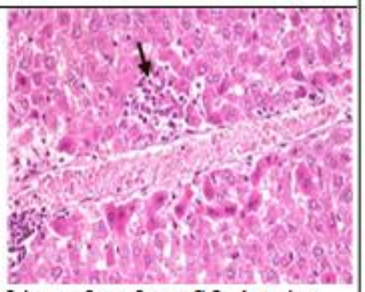
Figure 8: Photomicrograph (H & E X 400) of the liver for rat groups under study:



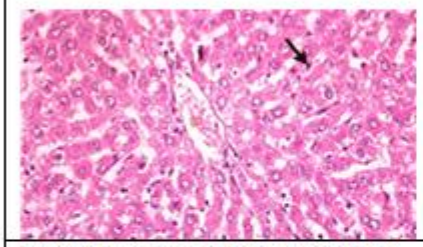
Liver of rat from G1 showing the normal histological structure of hepatic lobule



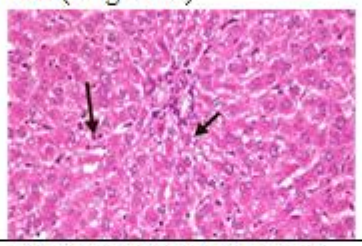
Liver of rat from G2 showing fibroplasia in the portal triad (short arrow) and portal infiltration with inflammatory cells (long arrow)



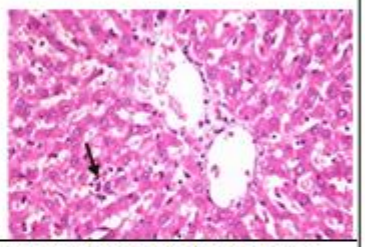
Liver of rat from G2 showing focal hepatocellular necrosis associated with inflammatory cells infiltration (arrow)



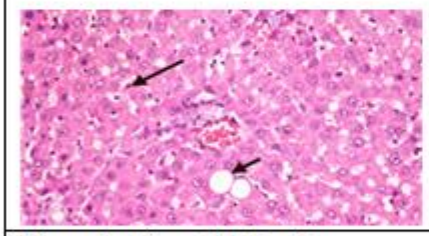
Liver of rat from G3 showing slight Kupffer cells activation (arrow)



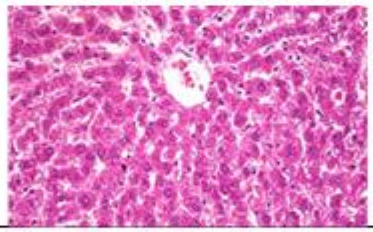
Liver of rat from G3 showing slight vacuolar degeneration of some hepatocytes (short arrow) and slight Kupffer cells activation (long arrow)



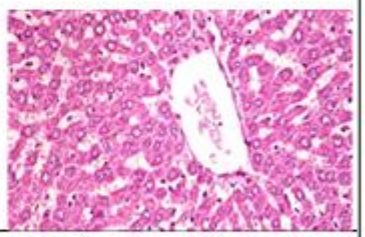
Liver of rat from G4 showing marked Kupffer cells activation (arrow)



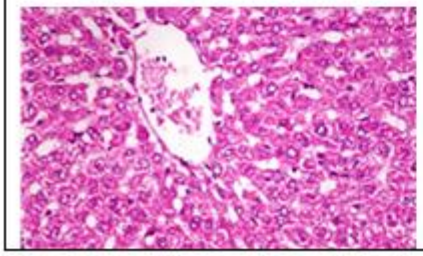
Liver of rat from G4 showing hepatocellular steatosis (short arrow) and Kupffer cells activation (long arrow)



Liver of rat from G4 showing no histopathological alterations

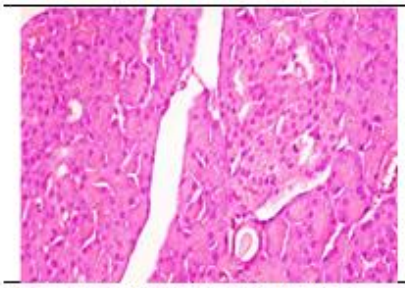


Liver of rat from G5 showing no histopathological alterations

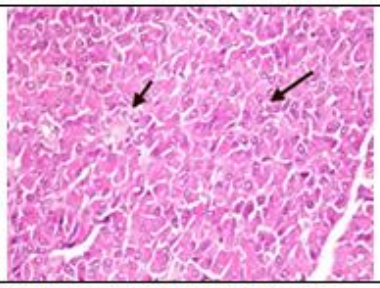


Liver of rat from G6 showing no histopathological alterations

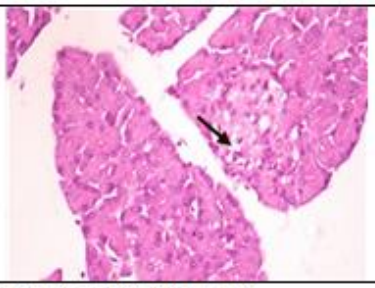
Figure 9: Photomicrograph (H & E X 400) of pancreas for rat groups under study:



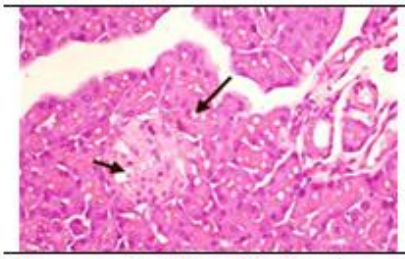
Pancreas of rat from G1 showing normal pancreatic acini and normal islets of Langerhan's



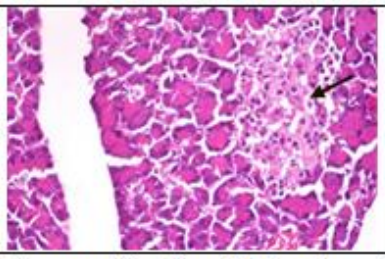
Pancreas of rat from G2 showing vacuolation of cells of islets of Langerhan's (short arrow) and vacuolation of cells lining pancreatic acini (long arrow)



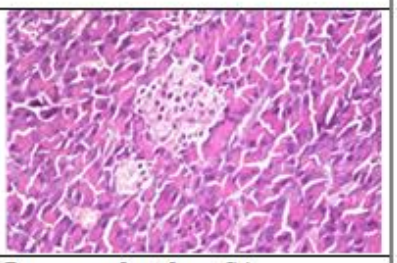
Pancreas of rat from G2 showing marked vacuolations of cells of islets of Langerhan's



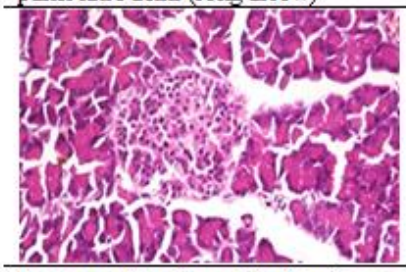
Pancreas of rat from G3 showing vacuolation of cells of islets of Langerhan's (short arrow) and vacuolation of cells lining pancreatic acini (long arrow)



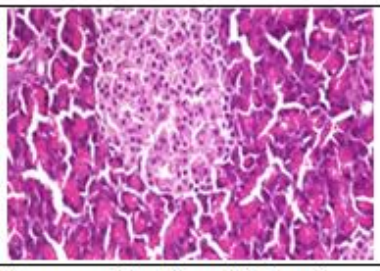
Pancreas of rat from G4 showing vacuolation of some cells of islets of Langerhan's (arrow)



Pancreas of rat from G4 showing no histopathological alterations

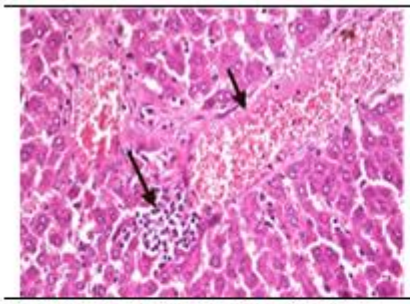


Pancreas of rat from G5 showing no histopathological alterations

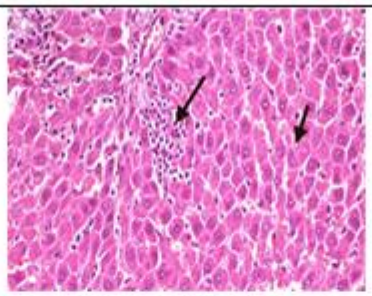


Pancreas of rat from G6 showing no histopathological alterations

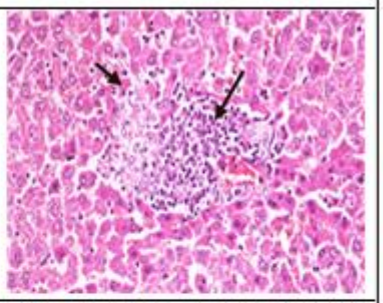
Figure 10: Histopathological changes in liver and pancreas were noticed for about 20% of the healthy rats supplemented with (5%) GBP:



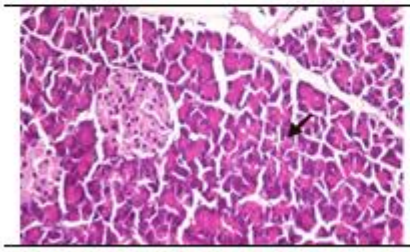
(a) Liver of rat from G5 showing congestion of central vein (short arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (long arrow)



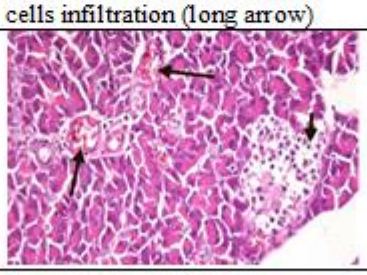
(b) Liver of rat from G5 revealing vacuolar degeneration of hepatocytes (short arrow) and focal hepatocellular necrosis associated with inflammatory cells infiltration (long arrow)



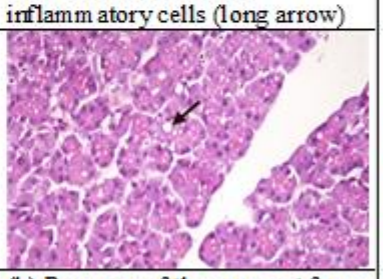
(b) Liver of the same rat from G5 demonstrating focal hepatocellular necrosis associated with inflammatory cells infiltration (short arrow) and portal infiltration with inflammatory cells (long arrow)



(a) Pancreas of rat from G5 showing slight vacuolation of cells lining some pancreatic acini (arrow)



(b) Pancreas of rat from G5 showing necrosis of cells of islets of Langerhan's (short arrow) and congestion of pancreatic blood vessel (long arrow)



(b) Pancreas of the same rat from G5 showing vacuolation of cells lining pancreatic acini (arrow)

Conclusion:

Oral administration of green banana powder as a dietary supplement improves biochemical and histopathological changes by controlling diabetic biomarkers, liver functions, and anti-inflammatory and antioxidant biomarkers. In terms of the amount consumed, 5 % GBP produced better results in healthy rats due to a balanced gut microbiome that allowed for better RS utilization. Whereas 2.5 % GBP produced better results in diabetic rats due to less available starch than 5 %, which aided in promoting the growth of beneficial bacteria over pathogenic bacteria, resulting in more conversion of RS to SCFAs. Increased GBP consumption, on the other hand, may have negative effects on the liver and other organs, as was indicated in figure (10), and may lead to diabetes.

Recommendations:

1) Consumption of GBP should be adjusted according to RS %.
2) Pilot study on diabetic and healthy human volunteers should be carried out.

Conflicts of interest

There are no conflicts to proclaim

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