

Antidiabetic Effects of Coffee Enriched with Maca and Marine Collagen Peptide (Blackbelt®) in a Type 2 Diabetes Mellitus Rat Model

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ABSTRACT

INTRODUCTION: The International Diabetes Federation (IDF) reported 463 million global cases of Type 2 diabetes mellitus (T2DM) in 2019. *Lepidium meyenii* (maca) and marine collagen peptide (MCP) have individually shown potential in alleviating T2DM symptoms, but their combined effects remain underexplored. This study evaluated the impact of Blackbelt® coffee, enriched with maca and MCP, on fasting blood glucose, insulin levels, and pancreatic and liver histology in a T2DM rat model. **MATERIALS AND METHODS:** Thirty-six male rats with a high-fat diet and streptozotocin-induced diabetes were treated with metformin, maca, MCP, Maca/MCP® (Blackbelt® formulation), or Blackbelt® coffee for 28 days. Fasting blood glucose (FBG) levels were monitored, and fasting serum insulin, HOMA-IR (insulin resistance), HOMA-B (β-cell function), and QUICKI (insulin sensitivity) were assessed. Histological analysis of the pancreas and liver was performed using haematoxylin-eosin staining. **RESULTS:** After 4 weeks, treatments significantly reduced FBG levels compared to control ($p < 0.05$), with Blackbelt® coffee notably increasing insulin production ($p < 0.05$). All groups showed decreased HOMA-IR ($p < 0.05$), and both metformin and Blackbelt® coffee groups had significant HOMA-B score increases ($p < 0.05$). Histological analysis revealed improved pancreatic health in all treated groups, with significant liver histology enhancement in the Blackbelt® coffee group. **CONCLUSIONS:** Blackbelt® coffee improved FBG levels, insulin resistance, and β-cell function more effectively than maca or MCP alone, and surpassed metformin in insulin production and hepatoprotective effects. Despite its promising potential for diabetes therapy, further research is needed to understand the synergistic effects of maca and MCP and the contribution of the coffee components.

Keywords

animal model, diabetes mellitus, *Lepidium meyenii*, maca, marine collagen peptide

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INTRODUCTION

Diabetes Mellitus (DM) is characterised by elevated blood sugar levels, known as hyperglycaemia. Type 2 Diabetes Mellitus (T2DM) is the most prevalent form, involving insulin resistance and insufficient insulin production.^{1,2} In 2019, the International Diabetes Federation (IDF) reported 463 million cases of T2DM worldwide, with 79% originating in low and middle-income countries. Southeast Asia, particularly Malaysia, is significantly affected, with Malaysia having 3.65 million cases.³ Projections indicate that diabetes prevalence will rise to 578 million by 2030 and 700 million by 2045.²

Hyperglycaemia is a major contributor to complications in organs such as the kidneys, liver, and central nervous system. Acute glucose level fluctuations increase oxidative stress, leading to tissue damage and systemic complications.⁴ Insulin resistance, marked by reduced tissue responsiveness to insulin, is central to these complications.⁵ Despite extensive research, there is a need for effective and affordable therapeutic interventions for T2DM. Functional foods and nutraceuticals are being explored as alternative treatments.

Epidemiological studies suggest coffee consumption offers health benefits, including a reduced incidence of T2DM, through potential regulation of glucose and lipid metabolism, and antioxidant and anti-inflammatory properties.^{6,7}

Lepidium meyenii, or maca, from Peru, contains bioactive compounds called macamides. These compounds have medicinal effects, potentially benefiting hyperglycemia and providing antioxidant properties that could reduce metabolic syndrome and oxidative stress.^{8,9} Marine collagen peptides (MCP) have been shown to improve glucose levels and insulin sensitivity in diabetes in both animal and human studies. These peptides enhance the insulin signaling pathway by reducing oxidative stress and inflammation, which are key contributors to insulin resistance.^{10,11}

Despite these promising findings, there remains a significant gap in translating these benefits into a practical, combined therapeutic approach. The current study aims to address this gap by evaluating a novel formulation, Blackbelt® coffee, which combines maca and MCP. Using a high-fat diet/streptozotocin (STZ)-induced diabetes rat model, the study investigates the effects of this formulation on biochemical parameters and histological changes in the pancreas and liver of T2DM rats. This research seeks to provide new insights into diabetes management, potentially offering a novel and effective approach to addressing the global T2DM burden.

MATERIALS AND METHODOLOGY

Drugs, chemicals, and supplements

STZ was obtained from Sigma Aldrich Company (St. Louis, MO, USA). Metformin (GLUCOPHAGE® 500 mg tablets) was from DoctorOnCall (Malaysia), licensed by Merck Santé France. Each Metformin tablet was dissolved in a 0.5% carboxymethyl cellulose solution.¹² Citrate buffer (pH 4.5) was from Evergreen Engineering & Resources (Selangor, Malaysia). Blackbelt® coffee, maca powder, and MCP powder were obtained from TheSpecialistsFormula Sdn. Bhd. (Selangor, Malaysia). Blackbelt® coffee is approved as safe by the Ministry of

Health Malaysia (FSQD 030685). Standard dry pellets were purchased from Gold Coin Sdn. Bhd. (Selangor, Malaysia). High-fat diet (HFD) comprises of commercially obtained 60% fat, 20% protein, and 20% carbohydrates.

Experimental animals

A cohort of 36 male Sprague Dawley rats, aged 7 weeks and weighing around 170 g, were obtained from A Sapphire Enterprise in Malaysia. After a one-week acclimatisation period, the rats were housed individually in polypropylene cages at the Physiology-Pharmacology Laboratory, International Islamic University Malaysia (IIUM) Kuantan Campus. The environment was controlled with a temperature of $28 \pm 2^\circ\text{C}$, $55 \pm 10\%$ humidity, and a 12-hour light/dark cycle. The rats were fed standard commercial pellets and given water ad libitum. All procedures adhered to IIUM Institutional Animal Care and Use Committee guidelines (Ethics approval ID: IACUC-2021-008) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011).

Study design

Figure 1 illustrates the general experimental workflow for the study conducted with 36 rats. After a one-week acclimatisation period, T2DM was induced using a combination of HFD and STZ. Rats were categorised as

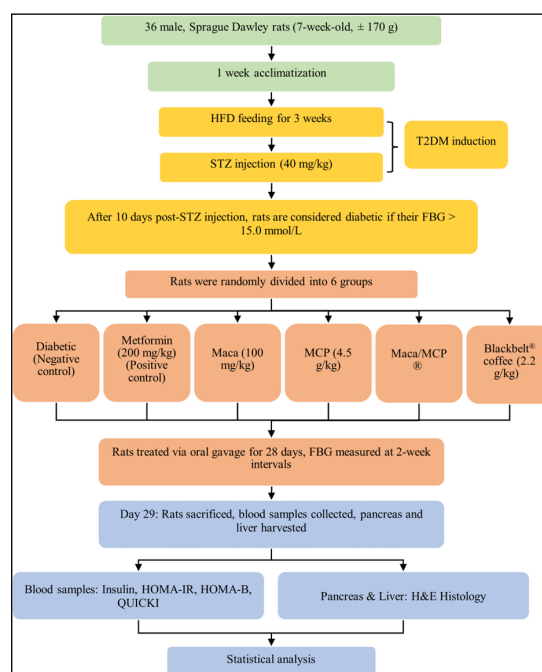


Figure 1: Study design

diabetic if their fasting blood glucose (FBG) levels exceeded 15.0 mmol/L. The rats were then randomly divided into one negative control and five treatment groups, and baseline FBG levels and weights were recorded. The treatment duration, doses of metformin, maca, and MCP were determined from previous studies with similar study designs, number of samples, and duration of study that have shown significant biochemical and histological results after 28 days of treatment.^{11,12,13} Group 5 received a combination of Maca/MCP® based on the formulated dose in Blackbelt® coffee. The dose for Blackbelt® coffee in Group 6 was calculated using an empirical method.^{14,15} In order to minimise the number of animals used as recommended by animal ethical guidelines, normal control (non-diabetic group) was omitted as the untreated diabetic group provided the necessary baseline for comparisons regarding the efficacy of different treatments. Extensive data on the physiological differences between non-diabetic and diabetic states is available from referenced studies.^{11,12,13}

Preparation of maca, MCP, and Blackbelt® coffee

Each Metformin 500 mg tablet was crushed and mixed in a 10 mL solution of 0.5% carboxymethylcellulose, then stirred to produce a suspension of 50 mg/mL.¹² Maca, MCP, Maca/MCP®, and Blackbelt® coffee were dissolved in distilled water. A solution containing 1 g of maca powder in 100 mL of distilled water was prepared to achieve a concentration of 10 mg/mL. Additionally, 300 g of MCP powder coffee was dissolved in 1 L of distilled water to yield a suspension of 300 g/L, while 100 g of Blackbelt® coffee was dissolved in 1 L of distilled water to create a suspension of 100 g/L. The formulation of Maca/MCP® cannot be disclosed but is lower than the Maca and MCP doses in the respective treatment groups. The dosage administered to the rats was determined based on the formulated dose of both maca and MCP present in Blackbelt® coffee. A magnetic stirrer and hotplate were employed during the preparation. Once the mixture reached the desired temperature, it was administered to the rats via an oral gavage feeding tube, ensuring accurate dosing.

Induction of T2DM

T2DM induction involved feeding rats a self-prepared HFD consisting of 60% fat, 20% protein, and 20% carbohydrates for 3 weeks, followed by a low-dose injection of STZ.^{12,16,17} STZ (40 mg/kg) was freshly prepared in 0.1 mM citrate buffer (pH 4.5) at a volume of 2 mL/kg and administered to rats via a single intraperitoneal injection.¹² Freshly prepared STZ was kept on ice before use.¹³ Treatment commenced upon confirmation of hyperglycaemia ten days post-STZ administration in the rats characterised by a stable hyperglycaemic state of FBG levels higher than 15mmol/L.¹²

Full blood glucose measurement

FBG for diabetic confirmation was taken at day 10 post STZ administration. FBG levels were monitored fortnightly using an Accu-Chek glucometer (Roche Diagnostics (Malaysia) Sdn. Bhd.).¹⁶ Blood samples were collected from the tail end and applied to glucometer strips.¹⁸

Insulin measurement

Upon completion of the treatment regimen on the 29th day, rats underwent an overnight fasting period and were subsequently anaesthetised using ketamine (80 mg/kg) and xylazine (10 mg/kg) the following day for blood sample collection from the retro-orbital region. The separation of serum from blood plasma was achieved through centrifugation at 3000 rpm for 20 minutes. Fasting blood insulin (FBI) levels were quantified using a rat insulin ELISA kit (EZRMI-13K, Merck KGaA, Darmstadt, Germany).^{12,17,19}

Measurement of HOMA-IR, HOMA-B, and QUICKI

Insulin resistance was assessed using HOMA-IR, calculated as $[FBG \text{ (mmol/L)} \times FBI \text{ (}\mu\text{U/mL)}] / 22.5$, with higher values indicating greater resistance.¹² β -cell function was evaluated with HOMA-B, computed as $20 \times FBI \text{ (}\mu\text{U/mL)} / [FBG \text{ (mmol/L)} - 3.5]$.⁴ QUICKI measures insulin sensitivity, with higher values indicating better sensitivity, calculated as $1 / [\log (FBI \text{ in mIU/L}) + \log (FBG \text{ in mg/dL})]$.⁴

Histological investigations

The pancreas and liver, rinsed with saline, were fixed in 10% neutral buffered formalin for 72 hours.¹⁷ Following fixation, the tissues were embedded in paraffin and sectioned at approximately 5 μm using a semi-automated rotary microtome (Leica Biosystems RM2245, United States) before mounting on glass slides.¹² The sections were then mounted in molten paraplast at 58°C–62°C. Subsequently, they underwent H&E staining, involving dehydration with ascending grades of ethyl alcohol (100%, 90%, and 70%) followed by clearing with xylene to remove the alcohol. After staining with H&E, the samples were examined under light microscopy (Eclipse E200-LED, Tokyo, Japan) at 200 $\times$ magnification.⁴

Pancreas histology

To assess the mean islet number, islets were counted in three distinct microscopic fields at 10 $\times$ magnification. The average number per field was then calculated for each study group. For measuring the maximum girth, a 1000 μm ocular grid at 10 $\times$ magnification was used to evaluate islet size. The maximum diameter was determined by comparing all available radii diameters for each islet and selecting the largest. This process was repeated in three separate microscopic fields, and the mean size of islets was computed for each group.^{20,21} The histological evaluation of pancreatic tissues involved a thorough examination of morphological features and pathological changes by a pathologist.

Liver histology

Analysis of liver tissue involved a comprehensive assessment of various parameters by a pathologist, covering overall liver morphology and specific indicators of pathological changes. This included examining general liver architecture, identifying fatty changes, detecting hydropic alterations, quantifying inflammatory cell infiltrates and vessel congestion, observing Kupffer cell hyperplasia, identifying haemorrhagic events, and detecting hepatocyte necrosis.

Statistical analysis

The results were presented as means $\pm$ SD and analysed

using SPSS Version 20 (SPSS Inc., Chicago, IL, USA). For variables measured over time, like FBG, repeated measures ANOVA with Bonferroni post hoc tests were used. For other variables such as insulin levels, HOMA-IR, and HOMA-B, one-way ANOVA was employed. Post hoc analysis (Duncan's test) followed if significant differences were detected. Serum insulin, HOMA-IR, HOMA-B, and QUICKI values in the T2DM rat model at week 4. Blackbelt® coffee had the highest serum insulin production of 46.19 ± 4.33 $\mu\text{IU/mL}$. It also reduced HOMA-IR (21.15 ± 3.93) and improved HOMA-B (8.07 ± 2.78).

RESULTS

Full blood glucose levels

Fig. 2 illustrates the mean and SD values of FBG for each group. The FBG levels consistently decreased across all groups from week 0 to week 4 following treatment administration. After 4 weeks of treatment with metformin, maca, MCP, or Blackbelt® coffee, a significant reduction in FBG levels was evident ($p < 0.05$). Compared to pre-treatment levels, FBG decreased by 63.52% (metformin), 47.09% (maca), 58.78% (MCP), and 47.97% (Blackbelt® coffee) respectively.

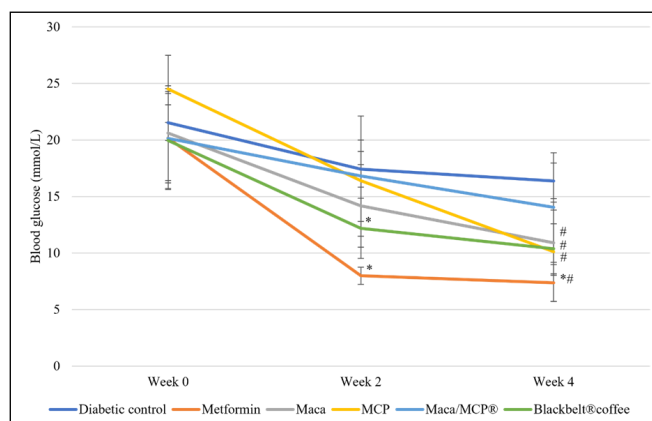


Figure 2: Changes in FBG levels in the different studied groups. The data were expressed as a mean $\pm$ SD ($n=6$). *significantly different when compared to the diabetic control group, $p < 0.05$, #significantly different when compared to week 0, $p < 0.05$.

Serum insulin levels, HOMA-IR, HOMA-B, and QUICKI scores

Table I shows serum insulin, HOMA-IR, HOMA-B, and QUICKI values in the T2DM rat model at week 4.

Blackbelt® coffee had the highest serum insulin production of $46.19 \pm 4.33 \mu\text{IU/mL}$. It also reduced HOMA-IR (21.15 ± 3.93) and improved HOMA-B (8.07 ± 2.78).

Table I Fasting serum insulin levels, HOMA-IR, HOMA-B and QUICKI scores of T2DM rat.

Groups	Parameters			
	Serum insulin ($\mu\text{IU/mL}$)	HOMA-IR	HOMA-B	QUICKI
Diabetic control	27.68 ± 6.66	31.58 ± 4.45	2.94 ± 2.08	0.26 ± 0.0058
Metformin	34.47 ± 1.44	11.27 ± 2.50^a	11.82 ± 6.16^a	0.27 ± 0.008^a
Maca	31.98 ± 8.62	15.48 ± 6.42^a	5.62 ± 3.48	0.27 ± 0.011
MCP	36.75 ± 7.50	17.23 ± 5.72^a	8.98 ± 5.85	0.27 ± 0.019
Maca/MCP®	28.66 ± 1.22^b	17.88 ± 7.40^a	5.74 ± 6.64	0.26 ± 0.018
Blackbelt® coffee	46.19 ± 4.33^{ab}	21.15 ± 3.93^a	8.07 ± 2.78^a	0.25 ± 0.005^b

The data were expressed as a mean $\pm$ SD (n=6). ^a: significantly different when compared to the diabetic control group, p<0.05. ^b: significantly different when compared to metformin, p<0.05.

Pancreas histology

Mean islet number and islet size

Table II shows a significantly higher islet count and size across all treated groups compared to the diabetic control group (p<0.05). While metformin showed the highest increases in both parameters, Blackbelt® coffee was a lose second.

Table II The mean number and diameter of pancreatic islets of studied groups.

Groups	Number of islets	Islet diameter (μm)
Diabetic control	9.33 ± 0.72	48.25 ± 1.37
Metformin	12.33 ± 0.61^a	80.08 ± 1.66^a
Maca	11.25 ± 0.32^{ab}	56.08 ± 0.88^{ab}
MCP	12 ± 0.27^a	58 ± 1.36^{ab}
Maca/MCP®	11 ± 0.38^{ab}	51.17 ± 0.88^{ab}
Blackbelt® coffee	12.25 ± 0.42^a	77 ± 1.19^{ab}

The data were expressed as a mean $\pm$ SD (n=6).^a: significantly different when compared to the control group, p<0.05;^b: significantly different when compared to the metformin, p<0.05.

Morphology of pancreas

Fig. 3 displays H&E-stained pancreatic images from different treatment groups, highlighting the effects of: A) Diabetic control, B) Metformin, C) Maca, D) MCP, E) Maca/MCP®, and F) Blackbelt® coffee. The pancreas of diabetic control group exhibited significant histological changes, as shown in Fig. 3A. Islet cell morphology

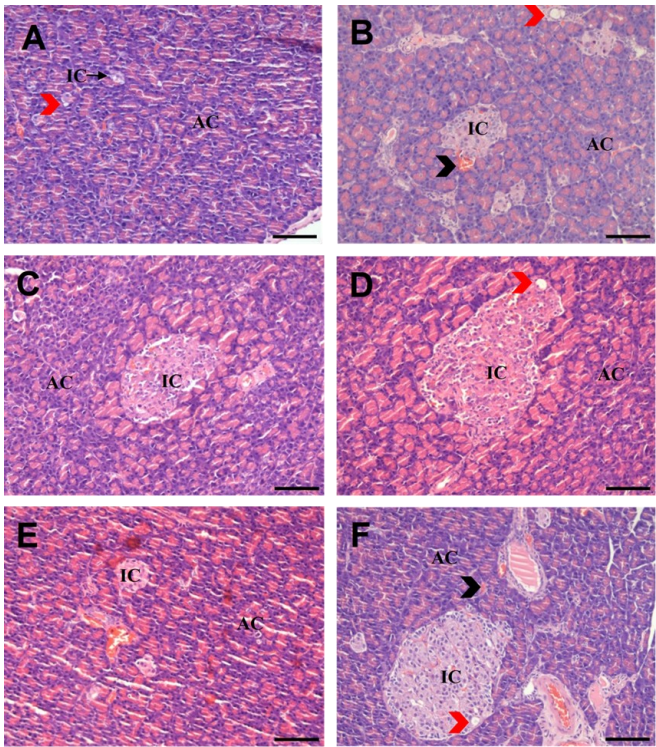


Figure 3: H&E-stained microscopic photomicrographs of the pancreas in the different studied groups at magnification: $\times 200$. A: Diabetic control, B: Metformin, C: Maca, D: MCP, E: Maca/MCP®, F: Blackbelt® coffee, AC: acinar cells, IC: islet cells, black arrow: congested blood capillaries, red arrow: vacuolation in cells. Scale bar: $200 \mu\text{m}$.

displayed a notable decrease in both number and diameter, indicating altered functionality and disrupted architecture. Additionally, pancreatic acinar cells showed signs of fat accumulation, along with inflammatory cell infiltrate and necrosis, suggesting tissue damage and inflammation consistent with T2DM.

In contrast to the diabetic control group, rats treated with metformin (Fig. 3B) showed distinct histological improvements in pancreatic morphology. Notably, there was an increase in the number and size of islets. Furthermore, reductions in fat accumulation and inflammatory cell infiltrate were observed in the pancreatic acinar cells, with less pronounced focal necrosis. Histological improvements were also noted in the pancreas of group receiving maca (Fig. 3C), MCP (Fig. 3D), Maca/MCP® (Fig. 3E), and Blackbelt® coffee (Fig. 3F) when compared to the diabetic control group. The pancreatic islets' morphology in rats treated with Blackbelt® coffee showed improvement comparable to those treated with metformin.

Liver histology

Morphology of liver

Fig. 4 displays H&E-stained liver images from different treatment groups, highlighting the effects of: A) Diabetic control, B) Metformin, C) Maca, D) MCP, E) Maca/MCP®, and F) Blackbelt® coffee. As shown in Fig. 4A, the liver histology of the diabetic control group displayed significant alterations, including fatty changes characterised by fat accumulation, cellular swelling indicating hydropic changes, and lymphocytic infiltration indicating inflammation. Moreover, signs of vessel congestion and Kupffer cell hyperplasia suggested a heightened immune response. Additionally, evidence of liver tissue haemorrhage and hepatocyte necrosis, reflecting cell death, were also observed. In contrast, metformin (Fig. 4B) groups did not show improvement in liver histology.

Maca (Fig. 4C) and MCP (Fig. 4D) groups exhibited improvements, displaying reduced fatty changes, hydropic changes, infiltration of lymphocytes, vessel congestion, Kupffer cell hyperplasia, and haemorrhage. Notably, MCP showed no hepatocyte necrosis. However, Maca/MCP®

(Fig. 4E) groups did not show improvement in liver histology.

Finally, Blackbelt® coffee (Fig. 4F) administration resulted in the most significant improvement in liver histology compared to other treatment groups. These included a reduction in fatty changes, indicating decreased fat accumulation within liver tissues, as well as a decrease in hydropic changes, signifying a reduction in cellular swelling due to water retention. Moreover, there was minimal infiltration of lymphocytes, suggesting reduced inflammation, along with less vessel congestion, indicating improved blood flow within liver vessels. Notably, no hepatocyte necrosis or Kupffer cell hyperplasia was observed, indicating the absence of liver cell death and normal proliferation of immune cells in the liver, respectively. Additionally, there was no evidence of haemorrhage, reflecting the absence of bleeding within the liver tissue. The liver tissue exhibited a more organised distribution of hepatocytes and maintained a typical hepatic architecture.

DISCUSSION

Effects of Blackbelt® Coffee on Biochemical Parameters and Indices

This model of streptozotocin (STZ) and HFD was chosen to closely mimic human Type 2 Diabetes Mellitus (T2DM). STZ damages pancreatic β -cells, reducing insulin secretion, while HFD induces insulin resistance, as validated by HOMA-IR and QUICKI assessments.^{17,22} This dual approach typically results in hyperglycaemia.^{23,24} The induced diabetic state in rats leads to metabolic dysregulation, including impaired glucose tolerance and altered lipid metabolism, manifesting as obesity and insulin resistance.²⁵

Maca and marine collagen peptide (MCP) individually demonstrated potential in lowering hyperglycaemia, and enhancing glucose regulation in T2DM, consistent with previous studies.^{12,14} However, the Maca/MCP® combination did not significantly reduce fasting blood glucose (FBG) levels in T2DM rats. In contrast, Blackbelt® coffee, which includes both maca and MCP, significantly decreased FBG levels, indicating its efficacy

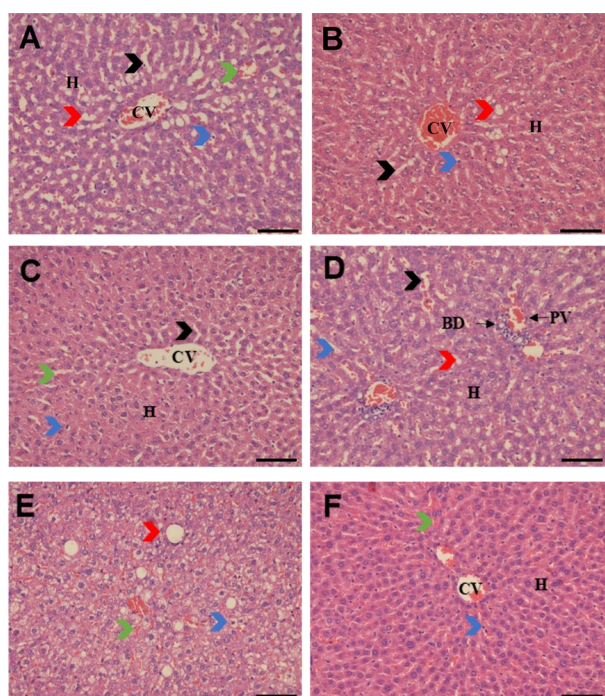


Figure 4 H&E-stained microscopic photomicrographs of the liver in the different studied groups at magnification: $\times 200$. A: Diabetic control, B: Metformin, C: Maca, D: MCP, E: Maca/MCP®, F: Blackbelt® coffee, CV: central vein, H: hepatocytes; PV: portal vein, black arrow: hepatic sinusoids, red arrow: vacuolated hepatocytes, green arrow: congestion, and the blue arrow: Kupffer cell. Scale bar: 200 μ m.

in managing hyperglycaemia.

The bioactive compounds in Blackbelt® coffee, such as caffeine and polyphenols, may synergistically enhance the effects of maca and MCP on glucose metabolism.⁶ The absence of coffee in the Maca/MCP® group might have impacted absorption rates and physiological responses, highlighting the importance of the delivery medium as the rich content of chlorogenic acid in coffee can enhance the absorption and bioavailability of other polyphenols.²⁶ Coffee consumption can also influence gut microbiota, affecting nutrient metabolism and absorption, potentially explaining the observed differences.²⁷ The results may also be due to the lower content of Maca and MCP in the Maca/MCP® formulation.

Blackbelt® coffee significantly increased insulin secretion and reduced fasting blood glucose (FBG) levels in rats with type 2 diabetes mellitus (T2DM), indicating enhanced insulin production. Treatments with maca, MCP, Maca/MCP®, and Blackbelt® coffee reduced HOMA-IR values from baseline, indicating decreased insulin resistance. In comparison, the HOMA-IR value in the Blackbelt® coffee group was higher than in the other treatment groups, likely due to elevated insulin levels. As this finding was not observed in the other groups, it is unlikely to be attributed to compensatory hyperinsulinaemia in response to insulin resistance. Rather, it is more likely the result of increased insulin secretion and pancreatic β -cell volume, driven by enhanced insulin signalling from caffeine, as shown in previous studies. Furthermore, caffeine's protective effects on pancreatic β -cells, through the alleviation of endoplasmic reticulum stress, may also play a role in this outcome.²⁸ Maca's effects on hormone modulation, antioxidant, and anti-inflammatory properties likely contribute to better glucose metabolism and reduced insulin resistance.^{29,30,31} Collagen peptides, including MCP, may enhance insulin action, decreasing HOMA-IR values, possibly through structural benefits to pancreatic tissues and improvements in gut health, which can influence insulin regulation and glycaemic control.^{32,33}

Blackbelt® coffee also exhibited elevated HOMA-B values compared to untreated diabetic rats, suggesting enhanced β -cell function. This improvement might be due to the bioactive properties of macamides in maca as well as collagen peptides and amino acids in MCP.^{32, 33, 34, 35}

Pancreatic protective effects

The induction of diabetes in animal models via a combination of HFD and STZ profoundly impacts the pancreas, as evidenced by histological examination. HFD contributes to increased fat accumulation and stress within the islets of Langerhans, while STZ induces diabetes by selectively destroying insulin-producing β -cells, leading to decreased insulin production and secretion.^{22,36} This combination accelerates diabetes development, resulting in significant loss of islet cells, disrupted islet architecture, and signs of inflammation and fibrosis.²²

Maca's anti-inflammatory and antioxidant properties help mitigate diabetes-related damage to pancreatic islets by preserving and potentially regenerating islets.³⁷ Maca's adaptogenic nature may also influence hormonal balance, crucial for pancreatic function and insulin secretion from islets.^{38,39} Similarly, marine collagen peptides (MCP) benefit connective tissues, potentially enhancing structural support for pancreatic tissues, including islets. Natural compounds in both maca and MCP may influence cellular proliferation and differentiation, targeting pancreatic progenitor cells or stimulating the growth of existing islet cells, thereby increasing islet count and size.²¹ However, the specific mechanisms behind these pancreatic islet changes in the context of diabetes are not well documented.

This study found that both the Blackbelt® coffee and metformin groups exhibited higher islet counts and larger islet diameters compared to other treatment groups. This suggests potential preservation or regeneration of pancreatic islets, which are crucial for insulin production and glucose regulation. The larger islet diameter indicates enhanced structural integrity and functionality.^{12,21} The comparable effectiveness of Blackbelt® coffee to

metformin further supports its potential as a natural intervention in diabetes management.

Hepatoprotective effects

Extended exposure to HFD, especially one rich in saturated fats, commonly precipitates non-alcoholic fatty liver disease (NAFLD), characterised by intracellular fat accumulation. This condition may advance to non-alcoholic steatohepatitis (NASH), which involves liver inflammation and cellular injury.³⁹ Streptozotocin (STZ), known for its toxic impact on pancreatic β -cells, can also indirectly influence the liver, causing systemic disruptions. The combination of HFD and STZ exacerbates liver-related issues, potentially leading to heightened hepatic steatosis, inflammation, and even fibrosis or cirrhosis.^{22,36}

This study revealed that metformin treatment did not yield significant improvement in liver histology, indicating its limitations in addressing the multifaceted nature of liver damage in diabetic rats. This result contradicts a previous study where metformin improved NAFLD.⁴¹ However, Blackbelt® coffee exhibited notable hepatoprotective effects, surpassing those of metformin. Hepatocytes exhibited a more organised distribution, indicative of typical hepatic architecture, and improvements were observed in hepatic sinusoids, suggesting enhanced vascular function.^{42,43,44} These findings collectively suggest that the unique components in Blackbelt® coffee including macamides and collagen peptides contribute to its hepatoprotective effects, potentially including anti-inflammatory or antioxidant properties. This is supported by previous studies that concluded treatment with maca improves liver enzyme levels and reduces malondialdehyde (MDA) levels in hepatotoxicity.⁴⁵ Additionally, a separate study found that MCP supplementation enhances hepatocyte viability and mitigates alcohol-induced hepatic steatosis.⁴⁶

CONCLUSION

In summary, Blackbelt® coffee has shown promising effects on various aspects of diabetes management, including lowering FBG levels, enhancing β -cell function, and potentially improving insulin resistance.

In this study, Blackbelt® coffee outperformed metformin in terms of insulin production and demonstrated additional hepatoprotective effects. However, further research is needed to understand the specific contributions of all its components.

LIMITATIONS OF STUDY

The limitations of this study include the absence of a normal (non-diabetic) control group. However, since the study focuses on the effects of maca, MCP, Blackbelt® coffee on a diabetic rat model, the untreated diabetic group served as the negative control, and the group treated with metformin served as the positive control. Another limitation is the absence of a group receiving coffee only. Including a coffee-only group in future studies could help distinguish the effects of the added ingredients from those of the coffee itself.

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