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Original Article

The Hyperglycemic and Anti-hyperlipidemic Properties of Honeybee Mixed with Wheat Germ in Streptozotocin-induced Diabetes in Rats

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ABSTRACT

Aim: This study aimed to find out the impacts of a honeybee and Wheat Germ (WG) combination on blood glucose and cholesterol concentrations in diabetic rats when administered orally. **Materials and Methods:** Thirty-eight male Sprague Dawley rats were divided into normal (*n*=8) and diabetic (*n*=30). The second group was injected with streptozotocin (at a dose of 75 mg/kg intraperitoneally), and those with blood glucose concentrations more than 250 mg/dL were classified as diabetic. Rats were divided into five groups, (1) non-diabetic group (*n*=8) received distilled water (2 mL/ day); (2) diabetic control group (*n*=8) received distilled water (2 mL/ day); (3) metformin diabetic group (*n*=7) received 2 mL of metformin drug solution (dose=100 mg/kg/day); (4) honeybee diabetic group (*n*=8) received honeybee solution (dose=2 g/kg/day); and (5) wheat germ diabetic group (*n*=7) received 2 mL of honeybee (95 g) mixed with wheat germ (5 g) solution (dose= 2 g/kg/day). After 28 days, blood was collected, and serum was extracted to determine glucose, HbA_{1c}, and blood lipids. **Results:** The results showed that honeybee alone and mixed with wheat germ increased blood glucose significantly above 350 mg/dL and HbA_{1c} above 8.4%. Conversely, the administration of wheat germ caused a noteworthy decrease in total cholesterol, triglycerides, and LDL concentrations among rats with diabetes. **Conclusion:** Consuming honeybees alone and combined with wheat germ may lead to hyperglycemia. Conversely, consuming honeybees in combination with wheat germ may result in hypocholesterolemia. These results indicate that honeybee and wheat germ could be functional food for managing hyperlipidemia in diabetic patients.

Keywords: Functional Food, Glucose, HbA_{1c}, Cholesterol, LDL, Metformin.

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INTRODUCTION

The increasing prevalence of Diabetes Mellitus (DM) stands out as one of the foremost afflictions and pressing issues of the contemporary era. DM is a persistent metabolic disease described by insufficient insulin levels and abnormal insulin secretion.[1] The prevalence of DM has experienced a notable escalation in recent years. As of 2019, it was projected that 9.3% (463 million) of the global population across all age cohorts had received a diagnosis of diabetes.[2]

Individuals who suffer from Type 1 DM (T1DM) and Type 2 DM (T2DM) have been consistently observed to exhibit elevated rates of Cardiovascular disease (CV).[3] Also, patients diagnosed with diabetes are at a significantly greater risk of evolving Atherosclerotic Cardiovascular Disease (ASCVD) than those without diabetes, with a four-fold increase in risk.[4] Furthermore, this CV risk increases as glycemic control worsens. Patients diagnosed with T2DM can be classified as having a moderate Cardiovascular (CV) risk.[5] Furthermore, it is noteworthy that over 33% of individuals with diabetes exhibit atherogenic dyslipidemia, thereby elevating their susceptibility to Atherosclerotic Cardiovascular Disease (ASCVD).[6] However, the presence of dyslipidemia in individuals with T2DM is accompanying with a notable rise in the occurrence of ASCVD and coronary artery disease.[7] To achieve a decline of 50% of the initial value, it is recommended that diabetic patients with coexisting lipid disorders lower their LDLc concentrations to below 70 mg/dL.[8]

The management of DM does not involve a panacea, so certain individuals with diabetes explore alternative and complementary therapies. (e.g., herbs, Chinese acupuncture, and functional

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foods). Within the realm of nutrition, the term "Functional Foods" pertains to food items that offer supplementary health benefits beyond their fundamental nutritional content. This statement highlights the strong correlation between health and the bioactive compounds present in these particular food items.[9]

Triticum aestivum L., commonly called Wheat Germ (WG), belongs to the Gramineae family and is recognized as the largest cereal-grass crop edible worldwide. The kernel of this crop is widely consumed as a staple food. Most wheat kernels classified as whole-grain consist of around 80% endosperm, 15% bran, and 5% germ.[10]

Wheat germ, scientifically known as *Triticum aestivum* L., is a residual substance obtained during wheat milling. The oil possesses a notable abundance of bioactive constituents, such as tocopherols, phytosterols, and policosanols, which are known to confer health benefits.[11] Numerous academic researchers have established the medicinal properties of WG and its oil, including antihyperglycemic and antioxidant benefits.[11,12] Chadha *et al.* (2015)[13] conducted a study that revealed that administering wheat germ oil to hyperlipidemic rabbits significantly reduced total serum cholesterol by over 90% and LDLc by over 95%, VLDLc by 53%, and triglycerides by up to 44%. Hassan and El Shafie (2022)[14] conducted a study that revealed that administering WG-supplemented chocolate to children with Down Syndrome (DS) caused a noteworthy reduction in blood total cholesterol, triglycerides, and LDLc. Another study carried out on pre-menstrual syndrome in women, revealed that the consumption of WG resulted in a significant decline in both physical and psychological symptoms and an overall decrease in the score.[15] While observing the study groups, it was observed that the severity of symptoms reduced in all groups. However, the group that was administered wheat germ extract exhibited a more notable decrease.

Several researches have indicated that honeybee has the ability to improve hyperglycemia and reduce oxidative damage associated with diabetes mellitus.[16,17] Additionally, honeybee has been reported to possess hepatoprotective properties by decreasing hepatic transaminases.[18] Limited research has been conducted to evaluate the impact of honeybee on glycated hemoglobin (HbA_{1c}), with findings indicating that honeybee consumption is associated with an increase in HbA_{1c} concentrations.[19-21] Previous research has indicated that co-administrating honeybee and anti-diabetic medications can enhance antioxidant properties and regulate blood glucose concentrations.[22,23] Furthermore, honeybee has been suggested to have potential benefits in managing diabetes mellitus by regulating blood glucose concentrations and mitigating the development of different metabolic disorders. In folk medicine, honeybees have been used to treat a range of ailments.[24]

The principal objective of this study was to find out the effectiveness of giving an oral mixture of wheat germ and honeybee (H-Wg) in reducing blood glucose, blood lipids, and histological alterations associated with STZ-induced T1DM in rats. The second study objective was to evaluate and compare the effects of wheat germ and honeybee combinations versus metformin on glycemia and lipidemia biochemical markers.

Hypothesis

H_0: The mixture made of wheat germ and honeybee does not decrease blood glucose and blood lipids.

H_A: The mixture made of wheat germ and honeybee decreases blood glucose and blood lipids.

MATERIALS AND METHODS

Rats

Fifty-eight male Sprague Dawley rats (n=58) weighing 150-170 g were obtained from Egypt's Ministry of Health's Animal Unit at Helwan Farm. For two weeks, the rats were kept in individual plastic cages under controlled environments, with a temperature of 22°C and a 12 hr light/dark cycle at the Faculty of Home Economics, Menoufia University, Egypt. Rats have unrestricted access to food and water. All experiments followed the National Institute of Health's Guiding Principles for Animal Care and Use. Rats were weighed after two weeks of acclimatization and randomly allocated to one of two groups: diabetic (50 rats) or normal (8 rats).

Induction of Diabetes (T1DM)

After two weeks of acclimatization of rats, type 1 diabetes mellitus was induced by intraperitoneal injections of Streptozotocin (STZ) as described previously.[25] The rats were injected with a dose of 75 mg/kg intraperitoneally of Streptozotocin (STZ) (Sigma-Aldrich, St. Louis, MO, USA). Following this, all rats fasted for 8 hr, and then blood samples were taken from the retro-orbital veins to determine blood glucose concentrations. The study included diabetic rats with blood glucose concentrations more than 250 mg/dL. Following the exclusion of rats with blood glucose concentrations below 250 mg/dL and deceased rats, 30 rats were included in the study and subsequently developed diabetes. In addition, diabetic rats were given 2 IU of human insulin (Glargine, Lantus) subcutaneously every week to keep them alive throughout the trial. To avoid spontaneous diabetes, blood glucose concentrations were also measured in the normal group.

Experimental Design

The study included all normal (8 rats) and diabetic (30 rats). In addition to the experimental procedure, all rats involved in this investigation were fed the standard diet. The proposed interventions were orally administered once per day. The weights

of the rats were also recorded, and diabetic rats have divided into experimental groups accordingly. The following were the experimental groups:

1- The non-diabetic group (ND-Gr) consisted of eight normal rats that received a daily 2 mL of distilled water orally per rat once daily.

2- The diabetic control group (DC-Gr) consisted of eight diabetic rats that received a daily 2 mL of distilled water orally per rat once daily

3- The metformin diabetic group (Mt-Gr), consisting of seven rats, received a daily oral 2 mL of metformin drug solution at a dose of 75 mg/kg body weight.

4- Honeybee diabetic group He-Gr; (8 rats) were orally treated once daily with 2 mL fruit honeybee solution (dose=2 g/kg body weight)

5- Honeybee mixed with wheat germ diabetic group HWg-Gr (7 rats) were orally treated once daily with 2 mL of honeybee (95 g) mixed with wheat germ (5 g) solution (dose= 2 g/kg body weight).

Diets

The animals were fed a standardized diet that contained 2850.0 kcal/kg. The diet was composed of 64.0% carbohydrates, 20.0% crude protein, 4.0% crude fat, 3.5% crude fiber, 6.0% ash, 0.50% salt, 1.0% calcium, 0.60% phosphorous, 20.0 IU/g Vitamin A, 2.20 IU/g Vitamin D, 70.0 IU/kg vitamin E, and trace elements such as cobalt, iodine, manganese, iron, copper, zinc, and selenium. The experimental procedures adhered to the guidelines outlined in the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Preparation of Honeybee Solutions and Metformin

Honeybees -fruit variety- (*Apis mellifera*) (H-Be) were obtained from local markets in Egypt. At the same time, Bob's Red Mill Company, USA, provided one bag (12 ounces) of roasted Wheat Germ (WG) natural raw grain (uncooked) (via Amazon).

The honeybee and wheat germ mixture (H-Wg) was made by mixing 5 g of wheat germ with 95 g of honeybee (ratio 95:5), which was then dissolved in distilled water (1:1). The total daily dose of the wheat germ was 50 mg/rat.

In addition, 1000 mg of metformin hydrochloride (CID, Company, Cairo, Egypt) was dissolved in distilled water (ratio 20mg/1 mL). Distilled water served as the vehicle treatment.

Determination of Chemical Compounds

The levels of Hydroxy Methyl Furfural (HMF), protein, and fat present in both honeybee and H-Wg samples were analyzed using the protocols established by the harmonized methods of the International Honey Commission and the Association of Official Analytical Chemists.[26,27]

The quantification of total phenols was conducted by means of colorimetry using the Folin-Ciocalteu reagent, following the methodology given by Singleton and Rossi in 1965.[28] The aluminum chloride colorimetric method, originally described by Ranganna in 1986, was used to determine the total quantity of flavonoids.[29]

Blood Sampling and Laboratory Analysis

After completing the intervention period spanning 28 days, blood samples were obtained from all rat groups following an 8 hr fasting period. The rats were euthanized under the influence of ether anesthesia. The hepatic portal vein was utilized to obtain blood samples, which were subsequently collected into tubes and subjected to immediate centrifugation at 3000 rpm for 10 min to facilitate serum separation. The serum was subjected to a precise aspiration process, followed by transfer into uncontaminated tubes, and subsequently preserved in a frozen state at a temperature of -20°C, in preparation for analysis. Except for glucose, which was promptly assessed in serum, all serum specimens were examined to ascertain HbA_{1c}, total cholesterol, triglycerides, HDLc, LDLc, and VLDLc.

Histopathological Examination

The pancreas and kidney specimens were isolated and submerged in a 10% neutral buffered formalin solution for 24 hr. Paraffin sections with a thickness of six micrometers were prepared and subsequently underwent staining with Hematoxylin and Eosin (H&E). The samples were then examined using light microscopy after 24 hr. The histopathological analysis was conducted at the histology laboratory in the Faculty of Veterinary Medicine, Cairo University, Egypt.

Statistical Analysis

The obtained data underwent statistical analysis and were presented in terms of the mean and Standard Deviation (±SD). The present study employed the statistical techniques of Analysis of Variance (ANOVA) and Least Significant Differences (LSD) to specify the degree of significance differences between various groups, with a confidence interval of 95%.

Ethical Approval

This experiment were ethically approved by the Scientific Research Ethics Committee (SREC), Faculty of Home Economics, Menoufia University, Egypt (08- SREC-07-2021).

RESULTS

Data presented in Table 1 indicates that the Hydroxy Methyl Furfural (HMF) concentration in honeybee and its blends were minimal, suggesting that the honeybees utilized in the research were fresh.

Wheat germ increases honeybees' water and protein content. Moreover, the honeybee wheat germ mixture exhibits a higher sucrose content of 24.6% compared to other samples. In contrast, when compared to the wheat germ and honeybee's mixture, the honeybees exhibited a higher concentration of fructose (37.5% compared to 17.3%) and glucose (29.5% compared to 16.4%).Regarding minerals and trace elements, the wheat germ and honeybee's mixture caused a substantial increase in their ash content (0.42 g/100 g vs. 0.09 g/100 g). Adding wheat germ to honeybees elevated sodium, potassium, phosphorus, calcium, and magnesium levels.The inclusion of wheat germ resulted in a three-fold increase in the total phenolic content of the mixture concerning its active compounds (41.90 mg/100 g vs. 13.93 mg/100 g). Concurrently, the augmentation of overall flavonoid content in wheat germ-honeybee mixture exceeded 12-fold compared to honeybee alone (21.3 mg/100 g vs. 1.69 mg/100 g).

Table 2 illustrates no statistically significant variations in the initial body weight across all groups, ranging from 194.0±5.6 g of Mt-Gr to 198.9±6.7 g of HWg-Gr. On the contrary, the final body weight of rats administered with wheat germ was higher than other groups examined. According to the data, there are statistically significant differences (p 0.05) between the groups exposed to wheat germ and honeybee's mixture and the diabetic control group. The HWg-Gr group exhibited a significantly (p<0.05) higher weight gain (60.7±12.2 g/28 days) than the negative and diabetic control groups (50.3±1.2 and 47.0±15.1 g/28 days, respectively).

The data shown in Table 3 demonstrate that the blood glucose concentration of the rats in the negative control group was measured to be 104.9±9.4 mg/dL. In contrast, all of the rats in the experimental group displayed readings that surpassed 250 mg/dL. When comparing the diabetic control group to the experimental groups, it was shown that the consumption of honeybee and honeybee mixed with wheat germ did not lead to a significant drop in blood glucose levels (358.1±18.4 and 373.0±21.9 mg/dL, compared to 369.1±12.9 mg/dL, respectively, at p>0.05). During the interim period, as compared to the normal and metformin groups, the diabetic control group and the rats fed with either honeybee or honeybee mixed with wheat germ exhibited the highest concentrations of HbA_{1c} (7.7±0.8, 8.7±1.1, and 8.4±0.9%, respectively, at a significance level of p<0.05). In contrast, metformin administration significantly reduced (p<0.05) blood glucose concentrations and HbA_{1c} levels.

According to Table 3, the total cholesterol concentrations of rats administered with wheat germ and honeybee's mixture were comparable to those of normal rats, with values of 80.4±7.3 and 76.0±8.8 mg/dL, respectively. Rats given only honeybees, metformin-treated rats, and wheat germ + honeybees showed a

Table 1: HMF, protein, fat, water, sugars, minerals, total phenols, and total flavonoids contents (per 100 g) of honeybee, the mixture of honeybee plus wheat germ (H-Wg).

Parameter	Honeybee	H-Wg
HMF (mg/100g)	0.42	0.185
Water (g/100g)	28.8	37.1
Protein (g/100g)	0.07	2.8
Fats (g/100g)	0.0	0.02
Sucrose (g/100g)	2.7	24.6
Fructose (g/100g)	37.5	17.3
Glucose (g/100g)	29.5	16.4
Ash (g/100g)	0.09	0.42
Sodium (mg/100g)	33.7	69.0
Potassium (mg/100g)	75.1	108.7
Iron (mg/100g)	0.23	0.29
Phosphor (mg/100g)	1.04	61.4
Calcium (mg/100g)	7.0	6.4
Zinc (mg/100g)	0.08	0.22
Chromium (mg/100g)	0.095	0.054
Cupper (mg/100g)	0.118	0.056
Manganese (mg/100g)	0.077	0.860
Magnesium (mg/100g)	0.27	15.84
Total Phenols (mg/100g)	13.93	41.90
Total Flavonoids (mg/100g)	1.69	21.30

Table 2: Effect of honeybee mixed with wheat germ (Wg) on body weight indices compared to rats treated with metformin (Mt-Gr) and normal control rats (ND-Gr) after 28 days of intervention in STZ-induced diabetic rats.

	ND-Gr (*n*=8)	D-Gr (*n*=8)	Mt-Gr (*n*=7)	He-Gr (*n*=8)	HWg-Gr (*n*=7)	ANOVA- value
Initial body weight (g)	195.8±11.2^{a}	196.5±7.2^{a}	194.0±5.6^{a}	195.3±2.4^{a}	198.9±6.7^{a}	0.437 (p=0.781)
Final body weight (g)	246.0±11.8^{ab}	243.5±21.6^{b}	245.0±6.8^{ab}	248.0±9.8^{ab}	259.6±18.5^{a}	1.362 (p=0.268)
Weight gain (g/28 day)	50.3±1.2^{a}	47.0±15.1^{a}	51.0±1.7^{ab}	52.8±7.9^{ab}	60.7±12.2^{b}	2.141 (p=0.098)

ND-Gr: negative control group; D-Gr: diabetic control group; Mt-Gr: Metformin diabetic group; He-Gr: honeybee diabetic group; and HWg-Gr: honeybee mixed with wheat germ diabetic group. Mean values subscribed with different letters in the same row show significant differences between those values as calculated statistically by ANOVA and LSD. * p<0.05, ** p<0.01, and *** p<0.001

Table 3: Effect of honeybee mixed with wheat germ (Wg) on fasting blood glucose (mg/dL) and HbA_{1c} (%), total cholesterol (TC) (mg/dL), triglycerides (TG) (mg/dL), HDL (mg/dL), LDL (mg/dL), and VLDL (mg/dL) in STZ-induced diabetic rats after 28 days of intervention, as compared to those of metformin-treated (M-T) and normal control rats (ND-G).

	ND-Gr (*n*= 8)	D-Gr (*n*=8)	Mt-Gr (*n*=7)	He-Gr (*n*=8)	HWg-Gr (*n*=7)	ANOVA-value
Glucose, baseline (mg/dL)	104.9±9.4^{a}	250.0±0.0^{b}	250.0±0^{b}	250.0±0^{b}	250.0±0.0^{b}	1784.8(p=0.000)
Glucose, final (mg/dL)	128.6±13.2^{a}	369.1±12.9^{b}	129.6±7.2^{a}	358.1±18.4^{b}	373.0±21.9^{b}	532.2(p=0.000)
HBA_{1c} (%)	4.8±0.2^{a}	7.7±0.8^{b}	4.7±0.9^{a}	8.7±1.1^{c}	8.4±0.9^{bc}	39.8(p=0.000)
TC (mg/dL)	76.0±8.8 ^{a}	97.4±12.0 ^{b}	88.3±14.0 ^{ab}	91.3±14.6 ^{bc}	80.4±7.3 ^{ac}	2.869(p=0.046)
TG (mg/dL)	67.5±12.6 ^{a}	115.4±9.0 ^{b}	65.3±10.8 ^{a}	71.8±8.1 ^{a}	63.6±19.7 ^{a}	15.6(p=0.000)
HDL (mg/dL)	41.3±6.3 ^{a}	40.0±2.2 ^{a}	39.2±2.5 ^{a}	38.5±10.6 ^{a}	42.0±3.7 ^{a}	0.319(p=0.862)
LDL (mg/dL)	21.2±5.9 ^{a}	34.3±11.1 ^{b}	36.1±11.2 ^{b}	38.5±8.7 ^{b}	25.7±5.9 ^{a}	4.03(p=0.013)
VLDL (mg/dL)	13.4±2.5 ^{a}	23.2±1.9 ^{b}	13.1±2.2 ^{a}	14.4±1.4 ^{a}	12.7±3.9 ^{a}	16.6(p=0.000)

ND-Gr: negative control group; D-Gr: diabetic control group; Mt-Gr: Metformin diabetic group; He-Gr: honeybee diabetic group; and HWg-Gr: honeybee mixed with wheat germ diabetic group. Mean values subscribed with different letters in the same row show significant differences between those values as calculated statistically by ANOVA and LSD. * p<0.05, ** p<0.01, and *** p<0.001

Table 4: Histopathological impacts of a combination of honeybee and wheat germ (Wg) on the kidneys and pancreas of STZ-induced diabetic rats after 28 days of treatment, in comparison to metformin-treated (M-T) and normal control rats (ND-G).

Histopathological lesion	ND-Gr (*n*= 8)	D-Gr (*n*=8)	Mt-Gr (*n*=7)	He-Gr (*n*=8)	HWg-Gr (*n*=7)
	Kidneys				
Necrobiosis of renal tubular epithelium.	-	++	-	+	-
Vacuolation of renal tubules.	-	++	+	+	+
Vacuolation of endothelial lining glomerular tuft.	-	++	-	+	+
Proteinaceous material in the lumen of renal tubules.	-	+++	-	-	-
	Pancreas				
Congestion of pancreatic blood vessels.	-	+	-	+	-
Necrosis of Langerhans's islets cells.	-	++	-	-	-
Vacuolation of Langerhans' islets' cells.	-	+	-	-	-
vacuolation of acinar epithelium.	-	++	-	-	+

(-) no change, (+) mild change, (++) moderate change, (+++) severe change.

considerable and highly significant reduction in total cholesterol concentrations compared to the diabetic control group.

Compared to the diabetic control group, diabetic rats fed wheat germ and honeybees mixture exhibited the lowest concentrations of blood triglycerides. Nevertheless, the rats subjected to wheat germ and honeybee's mixture and those undergoing metformin treatments demonstrated the lowest triglyceride concentrations.

Moreover, the administration of wheat germ and honeybee's mixture increased HDL concentrations, but there were no significant differences. The wheat germ and honeybee's mixture consumption exhibited the most significant effect on LDL concentrations in diabetic rats. The diabetic group that ingested wheat germ demonstrated the lowest LDL value among all the diabetic groups examined, and the outcomes were significant at $p<0.05$.

Table 4 shows that the group of rats with diabetes exhibited a moderate level of necrobiosis in the renal tubular epithelium and vacuolation in both the renal tubules and endothelial lining of the glomerular tuft in the kidneys. Concurrently, they experienced a significant accumulation of proteinaceous substances within the luminal space of the renal tubules.

In contrast, the honeybee group exhibited slight alterations, while the metformin and wheat germ groups demonstrated modest modifications. Regarding the pancreas, the histopathological analysis indicated moderate necrosis of the islets of Langerhans cells and moderate vacuolation of acinar epithelium in the diabetic control group. However, the administration of honeybee alone or in combination did not result in any significant or mild changes in the pancreatic tissues.

DISCUSSION

The concentration of HMF in the honeybee used in this study and its blends were minimal. However, the concentration of HMF present in honeybee serves as an indicator of its freshness. It can be affected by various factors such as storage duration, storage conditions, and thermal treatment. Elevated levels of HMF in honeybee can be attributed to adulteration, overheating, or prolonged storage.[30]

Adding wheat germ increases honeybees' water content slightly; however, honeybees containing a substantial amount of water are prone to fermentation, leading to a limited shelf life and an unpalatable taste, as noted by Bogdanov (2009).[27]

The results showed that when compared to the wheat germ and honeybee mixture, honeybees alone exhibited a higher concentration of fructose and glucose. It is well known that fructose does not exhibit any effect on the concentrations of blood glucose. in individuals with T2DM; in contrast, researches have revealed that it induces a minor increase in postprandial blood glucose concentrations among T1DM patients.[31,32] Moreover, as the blood glucose concentration increases, so does glucose concentration.[33] Nevertheless, multiple research studies have confirmed the elevated nutritional value of wheat germ.[34,35]

The results indicate that the consumption of wheat germ resulted in an enhancement of the body weight status. The rat group that consumed wheat germ significantly gained more body weight than the negative and diabetic control group ($p<0.05$).

In accordance with our research, Liu *et al.* (2022)[36] reported that the body weight of rats that were administered wheat germ was greater than the corresponding value of the control group ($p<0.05$). Meanwhile, Chadha *et al.* (2015)[13] conducted an animal study on rabbits that did not yield any statistically significant alterations in body weights after administering wheat germ.

The results showed that feeding honeybee and honeybee mixed with wheat germ significantly raised blood glucose and HbA1c concentrations above baseline values. By contrast, metformin administration produced a significant decline in both blood glucose concentrations and HbA1c.

These results indicate that honeybees had no impact on glycemic control, leading to a notable elevation in blood glucose concentrations. The present study's results are consistent with Ahmed *et al.* (2022),[21] who reported that feeding honeybees exacerbate blood glucose concentrations in STZ rats significantly. Al Aamri and Ali (2017)[37] conducted a study that revealed that the consumption of honeybee did not significantly impact body weight, glucose concentrations, or insulin concentrations. Abdul Sani and his colleagues (2014)[38] found that combining honeybee and ginger did not significantly impact blood glucose concentrations in STZ mice. The current study's results are in opposition to the findings of previous studies carried by Erejuwa *et al.* (2011),[22] Erejuwa *et al.* (2012),[18] and Nasrolahi *et al.* (2012),[23] which found that the administration of honeybee alone or in conjunction with metformin resulted in enhanced glycemic control. The dissimilarities noted between the current and those studies may be due to the type of honeybee utilized. Two distinct types of honeybee were utilized in the respective studies: Ilam honeybee and Malaysian honey. In contrast, the present investigation utilized the fruit variant of Egyptian honey.

While previous research has suggested that wheat germ and its oil may possess antihyperglycemic properties, as demonstrated in studies conducted by Singh *et al.* (2012),[39] Ghafoor *et al.* (2017),[11] and Liu *et al.* (2011),[12] the present study has found that the combination of honeybee and wheat germ had no effect on glycemic control and rats fed them had higher glucose levels when compared with negative control and metformin groups.

The findings of our study emphasized that wheat germ mixed with honeybee may benefit cholesterol and blood lipids in diabetic rats, demonstrating its potential as a functional food

for managing hyperlipidemia in individuals with diabetes and concurrent hypercholesterolemia.

The findings demonstrate a high level of consistency with the outcomes of Liu *et al.* (2022)[36] study, which revealed that administering wheat germ to hyperlipidemic rats for 28 days resulted in a significant decrease ($p<0.05$) in the concentrations of total cholesterol and LDLc in the serum. The researchers concluded that wheat germ has the potential to reduce cholesterol absorption in the intestine and subsequently reduce its concentrations in the serum.

A study conducted by Rezq and Mahmoud (2011)[40] to investigate the effects of wheat germ intake on rabbits with hypercholesterolemia. The results indicated a significant reduction in serum cholesterol concentrations, aligning with a separate experiment's findings where the experimental rats were fed a diet containing an additional 15% wheat germ. The experimental results indicate that the lipid profile of the rats exhibited a notable decrease, leading to a subsequent reduction in the occurrence of atherosclerosis.

Another research conducted by Park *et al.* (2015)[41] discovered that using wheat germ in treatment effectively prevented lipid accumulation in 3T3-L1 adipocytes. The findings suggest that this treatment may alleviate hypercholesterolemia.

Furthermore, a comparative study evaluated the impact of wheat germ oil and Atorvastatin medication, revealing that wheat germ oil exhibits protective effects against elevated lipid markers.[42] Additionally, the biologically active phytosterols present in wheat germ are crucial for reducing cholesterol absorption.[43]

Vitamin E is a prevalent lipid-soluble antioxidant, and wheat germ oil is a rich source of tocopherols, particularly Vitamin E. Antioxidants are possible antiatherogenic agents since they can reduce LDL oxidation and plaque formation, and antioxidant supplementation of the diet is thought to benefit the illness.[44] The potential anti-atherosclerotic impact of alpha-tocopherol in diet-induced hypercholesterolemic rabbits, as demonstrated by Schwenke *et al.* (2002),[45] suggests a possible connection between the hypocholesterolemic effect of wheat germ and its antioxidant properties.

CONCLUSION

The objective of this study was to evaluate the impact of a combination of honeybee and wheat germ on the levels of blood glucose and cholesterol in rats afflicted with diabetes. Streptozotocin was administered to induce diabetes in rats. Rats were deemed diabetic if their blood glucose levels exceeded 250 mg/dL. A wheat germ and honeybee mixture was orally administered to rats with diabetes, and its efficacy was compared to that of the drug metformin. The findings of this study suggest that wheat germ may positively impact body weight status, as evidenced by the observed increase in body weight among rats that were administered wheat germ. The administration of honeybee and honeybee mixed with wheat germ increased significantly the concentrations of blood glucose and HbA_{1c} above the normal and baseline values, inducing a state of hyperglycemia. The total cholesterol concentrations observed in rats administered with wheat germ were in the normal range and comparable to those of the control group and the results obtained from the experimental group were found to be similar to those of the control group. The study suggests that wheat germ administration can reduce cholesterol and blood lipids in diabetic rats. This finding indicates that wheat germ could be a functional food for managing hyperlipidemia in individuals with diabetes and comorbid hypercholesterolemia.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

WG: Wheat Germ; **DM:** Diabetes Mellitus; **CV:** Cardiovascular Diseases; **ASCVD:** Atherosclerotic Cardiovascular Disease; **T1DM:** Type 1 Diabetes Mellitus; **T2DM:** Type 2 Diabetes Mellitus; **DS:** Down Syndrome; **STZ:** Streptozotocin; **HMF:** Hydroxy Methyl Furfural; **ANOVA:** Analysis of Variance; **LSD:** Least Significant Differences; **SREC:** Scientific Research Ethics Committee.

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