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Nutritional and biological evaluation of *Vicia faba L.* green waste pods in streptozotocin-induced diabetic rats

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ABSTRACT:

This investigation aimed to evaluate the nutritional value and the effect of the pod's "byproduct" of green broad bean on glycemia and lipidemia in diabetic rats, in addition to their effect on liver enzymes and renal functions. Thirty male albino rats were classified into six groups (5 each). One of them was used as normal control, whereas the other groups received injections of streptozotocin to induce diabetes. One of these diabetic groups was saved as a positive control. The remaining diabetic groups received two concentrations of both pod powder (G3&G4) and pod extract (G5&G6). Sensory evaluation was carried out on a fried falafel fortified with green pods. The groups receiving the pod powder (10%) and the methanolic extract (250 and 500 mg/kg b.w.) showed notable reductions in serum glucose, HbA1C, TC, TG and LDL-c, liver enzymes, and kidney function tests. Significant increases were noticed in the treated groups' serum HDL-c and serum albumin. Histopathological examination revealed improvement in the pancreas in the treated groups. Sensory evaluation of the famous "fried falafel" product showed the best results with the paste fortified by 20%. The findings show that broad bean pods, typically wasted, could be a valuable source of nutraceuticals such as carbs, fiber, proteins, and amino acids. In conclusion, broad bean pods might serve as a promising adjunct therapy in treating diabetes and its consequences, enhancing the effectiveness of conventional treatments.

Keywords: *Vicia faba L. pods, Wastes, Antidiabetic, Histopathology and Falafel*

1- INTRODUCTION

Some phytochemicals with antioxidant activity can be found in vegetable waste [1]. Peels from fruits and vegetables are commonly thrown away or utilized as fertilizer and animal feed. These wastes have a strong propensity for microbial deterioration, which has serious negative effects on the environment. To achieve this, it is necessary to determine how to utilize these wastes profitably. Numerous studies are being conducted nowadays to find ways to use these wastes to lessen environmental pollution and get some health benefits [2].

Most people around the world use broad beans as a significant vegetable. The botanical name of the legume is Vicia

faba. The faba bean belongs to the Fabaceae family and gene group Vicia [3]. The fruit is additionally recognized as wide bean, horse bean, windsor bean, tick bean, fava bean, and other common names. V. faba is referred to as "kalamatar and bakala" in Hindi, the language of India [4]. There are four subspecies of it, according to Hossain & Mortuza [5], minor, equaine, major, and paucijuga. Since the V. faba fruit has been used for a very long time to cure disorders of the kidney, liver, and eye, those who regularly take it will experience more health benefits [6&7]. The unique quality of V. faba is that it is inexpensive and a quality protein source. The amount of protein was equivalent to that found in fish and meat.

It is hence commonly referred to as "poor man's meat" [8]. The fruit is a common source of protein-rich diet for people in the Mediterranean region, and it is also frequently used as animal feed due to its superior yield and nutritional value. One of the major crops in Egypt is V. faba, which is consumed by the populace in the form of soup, pastries, and cakes [9]. According to analytical data, high nutritional value can be found in the fruit of V. faba and is rich in dietary fiber, macronutrients, proteins, carbohydrates, folic acid, niacin, and vitamin C. The majority of the V. faba seed's seedy portion was rich in carbs (51-68%), followed by proteins (20-41%). The biologically enhanced antioxidant qualities of V. faba make it a powerful ally in the fight against conditions like colon cancer and diabetes mellitus [10].

The value of broad beans (Vicia faba L.) in the nutraceutical, functional, and economic realms has drawn considerable attention. Many people in both industrialized and developing countries depend on broad beans as a source of income, and they are seen to be a potentially inexpensive alternative protein source. The phenolic compounds, aminobutyric acid, vitamins, minerals, and dietary fiber found in large amounts in broad bean seeds are what give them their high levels of biologically active antioxidants and other nutrients [11&12]. Broad bean pods are a desirable source of high-quality components (particularly dietary fiber) that may provide various benefits intended for human application and raise the financial viability of this unused byproduct. Because of the antioxidant, anti-inflammatory, antimicrobial, and anticancer properties of green broad bean pods, raw or cooked fresh immature broad bean pods can be consumed by humans. Different bioactive components that could be used as additions in the preparation of functional foods can be obtained by solvent-extraction from dried, ripened pods [13]. Additionally, according to Mejri et al. [14], they contain a wealth of bioactive compounds that have antibacterial, antioxidant, enzyme-inhibitory, anti-diabetic, and health-promoting characteristics. The faba bean (Vicia faba

L.) is used in traditional remedies to lower cholesterol, such as antihyperlipidimic [15&16].

Dietary therapies have become increasingly important and extensively suggested for numerous disorders in the modern era. For example, individuals with cancer, heart disease, diabetes mellitus (DM), obesity, stroke, and heart disease should eat foods high in flavonoid chemicals to improve their health [17]. The polyphenols found in faba beans (Vicia faba L.) prevent the digestive enzymes glucosidase and lipase from breaking down fats and carbohydrates, respectively. A decreased postprandial glycemic response may result from glucosidase activity inhibition, which can restrict the absorption of sugar and the digestion of carbs. As a result, it has been acknowledged that inhibiting glucosidase is a therapeutic target for controlling postprandial hyperglycemia. Lipase activity suppression might decrease the absorption of fat [18]. Dietary fibers are an alluring source of valuable nutrients that may provide various benefits for human health and boost this underutilized by-product's profitability [19&20].

Vegetables are a common key element in many recipes for fried cuisine. Falafel, a fast, Middle Eastern cuisine snack, is one of the most well-known fried vegetables. It goes by a number of names that vary depending on where you are. It is a little fried ball made from faba beans and is referred to as "Ta'amiyya" in Egypt and Sudan [21]. It is perceived as a meal eaten for breakfast or dinner, particularly in families with modest incomes where it is taken in large quantities due to its affordable price, constant availability, and unique flavor [22]. Broad beans, a widely consumed crop grown around the world, are marketed via a straightforward commercial method of removing pods. Broad bean pod (BBP), a legume byproduct, and other byproducts may be significant sources of dietary fiber [23]. Some nutritional advantages are linked to the lower rate of starch digestion in legumes and the presence of dietary fiber in legumes, which is primarily found in their husk fractions [24]. Therefore, to add these advantageous features to various foods, this byproduct could be used.

In this study, we attempted to assess the nutritional value and the effect of broad bean pods on diabetic rats. In addition to their effect on lipid profile, liver enzymes and kidney functions.

2- Materials and Methods

2.1. Materials:

Broad bean: Green broad bean (Vicia faba L) was obtained from a local market in Mansoura City. The plant collection and use followed all the relevant guidelines.

Chemicals: Elgomhoria Company in Mansoura City, Dakahlia Governorate, Egypt, was the source of all chemicals and kits purchased.

Streptozotocin (STZ) was purchased from El-Gomhoria Company in Cairo, Egypt, for chemicals and drugs.

Animals: The Agriculture Research Centre in Giza, Egypt, purchased thirty male albino rats "Sprague Dawley rats ", each weighing 135±5g.

Ethics approval and consent to participate

The protocol of this study was approved by the research ethics committee of the Faculty of Specific Education at Mansoura University (code 19: 11-2022). Biological experiment was carried out in compliance with globally recognized criteria for the care and use of laboratory animals, as well as the ARRIVE recommendations (https://arriveguidelines.org).

2.2. Methods:

2.2.1. Broad bean pods powder preparation:

The broad beans (Vicia faba L.) were scrubbed carefully and given a thorough water wash. Following the seeds' removal, the green pods were dried in an oven at 40 °C until their weight remained constant, pulverized into a finely ground powder, and stored at - 20 °C, as shown in photo. 1.

Photo1: Broad Bean Pods Powder Preparation

2.2.2. Producing a methanolic extract from broad bean pods:

250 g of powdered broad bean pods (Vicia faba L.) were soaked in 1L of methanol, mixed thoroughly, and allowed to stand overnight before being filtered through paper. The filtrate was then stored in a dark bottle. The residue was mixed well with more methanol, agitated overnight, and the filtrate from that process was added to the first filtrate. The residue was removed after a further overnight soak in methanol. To generate the methanolic extract solution, all three filtrates were combined. A rotary evaporator was used to evaporate the solvent out of the mixture. The resultant extract was gathered and stored in dark vials until use.

2.2.3. Preparation of product " Falafel":

According to Saba [25], the components of falafel are listed in Table (1). The broad bean pods were not included in the preparation of the control sample. The whole broad bean pods with seeds and the pods without seeds were added to the paste of falafel at 20, 30, and 40% after being thoroughly cleaned and rinsed in water. After dehulling and soaking in twice as much water at room temperature for 12 hours, the faba beans were drained. Following the addition of the onion, food salt, parsley, coriandrum, dill, and spices, the mixture was mixed for 20 minutes in a Kenwood mixer until it was sufficiently smooth and uniform to form balls (15 g each), which could subsequently be fried for 4 minutes at 170°C in a mixture of soybean and sunflower oil.

Table 1: Falafel components with broad bean pods (the whole broad bean or with the waste pods only)

Formation (g)	Control (g)	Broad bean pods Falafel		
		20%	30%	40%
Dehulled beans	100	100	100	100
Broad bean pods	0	20	30	40
Onion	30	30	30	30
Parsley	10	10	10	10
Coriandrum	10	10	10	10
Dill	10	10	10	10
Spices	1	1	1	1
Food salt	1	1	1	1
Total	162	182	192	202

2.2.4. Chemical analysis:

- Moisture, ash, fat, fiber and total protein contents were completed in compliance with the guidelines of the A.O.A.C [26]. Carbohydrate content was calculated by the difference by the equation: 100 – (ash% + moisture %+ fat %+ protein%)
- Fractionation and identification of the amino acids were carried out by using an automatic amino acid analyzer S 433 [27].

2.2.5. The basal diet:

The method of the National Research Conical [28] was followed in the preparation of the basic diet. International standards for the care and management of laboratory animals were followed in all biological studies.

2.2.6. Induction of diabetes:

By administering STZ (30 mg/kg body weight) intraperitoneally (i.p.) twice, diabetic rats were produced. Three days separated the first injection from the second. Injecting STZ twice caused the islets of Langerhans to undergo gradual destruction of **β**-cells, raising blood glucose levels in the rats as a result [29]. The rats were deemed diabetic after three days if their fasting blood glucose level was greater than 200 mg/dl [30].

2.2.7. Experiment design:

The rats were left for a weak on basal diet and water ad libitum for adaptation. After the adaptation period, rats were classified into six groups (five rats each), one of them remained on the basal diet only and served as the normal control (Group1). The rest of the 5 groups were intraperitoneally injected with STZ at dose of 30 mg/kg body weight twice to induce diabetes. One of these diabetic groups remained on the basal diet and was considered a positive control (Group2). The other four diabetic groups were treated with the powder and extract of broad bean pods as follows:

Group 3: Fed on a diet containing broad bean pods powder at a concentration of 5%.

Group 4: Fed on a diet containing broad bean pods powder at a concentration of 10%.

Group 5: Received broad bean pods extract daily in an oral dosage of 250 mg/kg b.wt. through a stomach tube.

Group 6: Received broad bean pods extract daily in an oral dosage of 500 mg/kg b.wt. through a stomach tube.

All rats were euthanized after 28 days. Rats were anesthetized by injecting ketamine (50 mg/kg) and Lidocaine HCl (20 mg/ml) i.p. The rats were then laparotomized and euthanized by whole blood collection from the inferior vena cava in clean tubes , left for 10 minutes and then centrifuged at 4000 rpm/min to isolate serum. The pancreas organ was isolated for histopathology analysis.

2.2.8. Biological estimations:

2.2.8.1.Body weight gain (BWG) and feed efficiency ratio(FER): They were calculated according to Chapman et al. [31].

$$\text{BWG \%} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

FER = BWG (g) / Feed intake daily (g)

2.2.8.2. Biochemical analysis of serum:

* HbAlc % was determined according to Abraham et al. [32].

- Serum glucose level was determined by an enzymatic method according to Kaplan [33].

- Serum total cholesterol(TC) was estimated based on Thomas [34].

- Serum triacylglycerol was estimated based on Fossati and Prencipe [35].

- The measurement of high-density lipoprotein (HDL-c) followed the procedures laid out by Warnick et al., [36].

- The following equation, according to Friedewald et al. [37], was used to determine LDL-c. LDL-c = TC – (HDL-c + TG/5).

2.2.8.3. Liver function was determined as following:

The procedure outlined by Burtis et al. [38] was followed to test the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The method developed by Doumas and Biggs [39] was used to measure serum albumin.

2.2.8.4. Kidney function was obtained as the analysis shown below:

The procedures outlined by Fassati et al. [40] and Young [41] were used to measure

serum uric acid and creatinine, respectively.

2.2.8.5. Histopathological study:

- Pancreas samples were obtained from the autopsies of the sacrificed rats. Samples were fixed in 10% formalin saline solution for 10 hours [42], and then rinsed in tap water for 12 hours. The tissue samples were dehydrated using serial alcohol concentrations. After being cleaned in xylene, tissue samples were paraffin-embedded. By using a sliding microtome, the paraffin blocks were divided into sections of three microns. For light microscopy, the acquired tissue sections were assembled on glass slides and stained with hematoxylin and eosin.

2.2.8.6. Sensory evaluation

25 panelists from the faculty, staff, and students of the Faculty of Specific Education at Mansoura University evaluated the sensory properties of the samples by Aljdely and Hemida [43]. Taste, color, consistency, odor, and overall acceptability, which were calculated as the sum of all the sensory metrics' mean scores were the parameters. The sensory characteristics were rated on a 9-point hedonic scale, with 1 representing extreme dislike and 9 representing extreme liking.

2.2.9 Statistical analysis:

The gathered information was displayed as means and standard deviations. SPSS, version 24, a computer program for statistical analysis, was used to complete all tests [44].

3- RESULTS AND DISCUSSION

The data in Table (2) showed the percentage of chemical composition content of broad bean (Vicia faba L.) pods. Data recorded were 9.27, 8.375, 0.38, 7.22, 14.59 and 74.75 for moisture, protein, fat, ash, fibers and total carbohydrates, respectively. Broad bean pods have a high moisture content (79.26%, wet weight basis), as shown by Elbadrawy and Mostafa [13]. Proteins, carbs, fats, and dietary fiber all had average values of 13.81, 18.93, 0.92, and 57.46% (dry weight basis). Additionally, Vernaleo [45] noted that fava beans have a high protein content, are rich in dietary fiber, and contain phytonutrients such as plant sterols and isoflavone. Furthermore, it has a lot of folates. There have also been reports of significant concentrations of pyridoxine, vitamin B6, thiamin, riboflavin, and niacin.

Table 2: The chemical contents of dried broad bean pods (g/100g)

Components %	Moisture Mean±SD	Protein Mean±SD	Fat Mean±SD	Ash Mean±SD	Fibers Mean±SD	Carbohydrates Mean±SD
Broad bean pods	9.27±0.08	8.38±0.06	0.38±0.43	7.22±0.10	14.59±0.03	74.75±0.16

SD: Standard deviation.

Data in Table (3) demonstrated that broad bean pods are rich sources of essential amino acids, which raise their nutritional value. Threonine recorded the highest

amount of essential amino acids as its value reached (3.726 g/100g), followed by histidine (0.606 g/100g). On the other hand, aspartic acid recorded the highest amount of non-essential amino acids (1.088 g/100g), followed by glutamine (0.76 g/100g), while, cysteine and serine were not detected in the pods. The obtained results were close to those of Al- Gaby [46] who found that faba meal protein has nearly twice the concentration of aspartic acid recorded for corn meal protein. In the protein found in faba meal, lysine is present in large levels. This result has been confirmed by many workers [47].

Table 3: Amino acids content of broad bean pods g/100g sample

Amino acids (g/100g sample)			
Essential		Non Essential	
Histidine	0.606	Alanine	0.4
Isoleucine	0.056	Aspartic acid	1.088
Leucine	0.472	Cysteine	N.D
Lysine	0.062	Glutamine	0.76
Methionine	0.044	Glycine	0.602
Phenylalanine	0.214	Proline	0.212
Threonine	3.726	Serine	N.D
Valine	0.098	Tyrosine	0.352
Lysine	0.062	Ammonia	N.D

N.D: Not detected

It has been shown in Table (4) the influence of broad bean pods powder and its alcoholic extract on body weight gain (BWG), feed intake (FI) and feed efficiency ratio (FER) in diabetic rats at the end of the experiment. Significant decreases ($p<0.05$) in body weight gain (8.28±1.20 %), feed intake (21.39±0.26 g/day) and FER (0.014±0.002) were observed in diabetic control (+ve control) compared to the normal control group (-ve control). BWG recorded 8.85±4.30, 16.97±4.11, 14.09±1.79 and 12.87±4.18 %, while FI scores were 21.21±0.32, 22.48±0.36, 21.77±0.27 and 21.58±0.23 g/day in diabetic rats groups treated with broad bean pods powder (5 and 10) % and broad bean pods alcoholic extract in an oral dosage of (250 and 500) mg/kg b.wt., respectively. Meanwhile, the FER recorded was 0.015±0.007, 0.027±0.006, 0.023±0.003 and 0.021±0.007, respectively. Thus, it was evident that the administration of powdered broad bean pods in the diet (5 and 10%) and broad bean pods alcoholic extract orally at doses (250 and 500) mg/kg to the diabetic rats for four weeks caused a significant increase ($p<0.05$) in body weight gain and FER compared to the control group of diabetics. According to Howarth et al., [48], soluble or insoluble fiber consumption promotes post-meal satiety and reduces subsequent hunger under fixed energy intake. Furthermore, this review indicated that high-fiber diets

reduce calorie intake and body weight, at least for short-term follow-up to avoid obesity and overweight [49]. While rats were fed a 15 % mixture of fruits peels fortified with a basal diet, significant increases compared with the positive group was achieved in fthe eed efficiency ratio [50].

Table 4: Influence of broad bean pods powder and extract on body weight gain and feed efficiency ratio of STZ diabetic rats

Groups	Initial weight Mean±SD	Final weight Mean±SD	BWG % Mean±SD	FI Mean±SD	FER Mean±SD
Control (-)	199.00±4.58a	250.00±4.00 a	25.70±4.86a	23.08±0.43 a	0.040±0.007 a
Control (+)	201.67±4.16a	218.33±2.52d	8.28±1.20c	21.39±0.26 c	0.014±0.002c
Powder 5%	201.00±4.58a	218.67±4.51d	8.85±4.30c	21.21±0.32 c	0.015±0.007 c
Powder 10%	200.67±2.52 a	234.67±5.69 b	16.97±4.11b	22.48±0.36 b	0.027±0.006 b
Extract 250	198.67±2.52a	226.67±4.73c	14.09±1.79bc	21.77±0.27 c	0.023±0.003bc
Extract 500	203.00±5.0 a	229.00±3.61bc	12.87±4.18 bc	21.58±0.23 c	0.021±0.007bc
LSD at 0.05	ns	7.63	6.54	0.56	0.010

Significant variations in the results (p<0.05) are shown by different superscripts in the same column (a, b, c, and d); LSD stands for least significant variations; SD: Standard deviation of means; ns= non-significant; BWG: body weight gain; FI: feed intake; FER: feed efficiency ratio

Blood glucose and HBA1C in the investigated groups are shown in Table (5). Diabetic control rats (+ ve) had a significant increase (p<0.05) in blood glucose (438.33 ± 11.50 mg/dl) and HbA1C (4.55 ± 0.19 %) compared to normal control (-ve control). Administration of broad bean pods powder (5 and 10)% in the diet and broad bean pods alcoholic extract orally in an oral dosage of 250 and 500 mg/kg. bw to STZ diabetic rats for four weeks significantly reduced (p<0.05) the elevated blood glucose and HbA1C gradually by increasing the powder and alcoholic extract doses in comparison with those of positive control rats. The best result in decreasing blood glucose was achieved in the group receiving 500 mg/kg broad bean pods alcoholic extract (134.33 ±14.50 mg/dl), followed by the group of 10% broad bean pods powder (152.67±7.51 mg/dl), then the 250 mg/kg broad bean pods extract group (196.33 ±12.10 mg/dl); finally, the 5% broad bean pods powder group (259.00±8.00) as compared to the positive control group (438.33 ± 11.50 mg/dl) at P<0.05.

The main reasons for the rise in blood glucose in response to STZ induction, according to Mejri et al. [14], were the excessive synthesis of glucose in the liver and the selective degradation of islets of Langerhans pancreatic cells, which are indicative of hormonal imbalances and metabolic disorders. The hull of broad beans is a good source of non-soluble fiber and has the ability to boost food fiber concentrations by up to 15g/100 g. Additionally, it has a higher concentration

of polyphenols and antioxidants, slows down the activity of the enzymes -amylase and -glucosidase, and may be able to lower the glycemic index of foods [51]. Because of their positive effects on the digestive system, ability to avoid chronic diseases, and enhancement of glucose tolerance in diabetics, broad beans with a high fiber content are recommended for intake [52]. Additionally, Sello [53] noted that all of the groups treated with plant extracts, together with the control group, confirmed a significant decrease in blood glucose levels at 2, 4, and 6 hours after extract administration .

Table 5: Influence of broad bean pods powder and extract on blood glucose of STZ diabetic rats

Groups	Glucose (mg/dl) Mean±SD	HBA1C (%) Mean±SD
Control (-)	81.33±3.51f	2.76±0.16e
Control (+)	438.33±11.50a	4.55±0.19a
Powder 5%	259.00±8.00b	4.14±0.21b
Powder 10%	152.67±7.51d	3.36±0.18d
Extract 250	196.33±12.10c	3.81±0.18c
Extract 500	134.33±14.50e	3.13±0.14d
LSD at 0.05	18.10	0.32

Significant variations in the results (p<0.05) are shown by different superscripts in the same column (a, b, c, and d); LSD stands for least significant variations; SD: Standard deviation of means.

As shown in Table (6), when compared to rats fed only on a basal diet (a normal control), STZ diabetic rats had significantly higher serum levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL-c), while there was a significantly lower serum level of high-density lipoprotein (HDL-c). In comparison to positive control rats, the elevated serum levels of TC, TG, and LDL-c were significantly reduced (p<0.05) after four weeks of feeding diabetic rats 5 and 10% broad bean pod powder and broad bean pod alcoholic extract in an oral dosage of 250 and 500 mg/kg to diabetic rats, the best results of lipid profile were achieved in the rats group which fed 500 mg/kg broad bean pod alcoholic extract. The represented data was consistent with the findings of Mejri et al. [14], who discovered a substantial (p<0.05) increase in lipid parameters, such as TC, TG, and LDL-c, with the exception of HDL-c. As opposed to the normal control mice, the alloxan-diabetic mice had lower serum albumin contents. In several animal models, these observations have been characterized as characteristic symptoms and the main molecular indicators of diabetes caused by streptozotocin or alloxan [54&55]. The altered lipid profile was primarily caused by an increase in lipase activity with a concurrent cholesterol biosynthesis through the activation of their corresponding enzyme 3-hydroxy-3-methyl-methylglutaryl-coenzyme-A [56&57]. The fact that the aforementioned parameters were

significantly reduced after being given the BBP extract orally suggests that it has a lipid-lowering impact on diabetic animals. The primary cause of the BBP extract's ability to decrease cholesterol may be its inhibitory effect on lipid metabolic enzymes and/or its potential ability to prevent lipid peroxidation [14].

Table 6: Influence of broad bean pods powder and extract on lipid profile in STZ diabetic rats

Groups	TC (mg/dl) Mean±SD	TG (mg/dl) Mean±SD	HDL-c (mg/dl) Mean±SD	LDL-c (mg/dl) Mean±SD
Control (-)	62.33±9.07d	74.00±6.56c	44.33±7.09a	3.00±1.00e
Control (+)	120.67±3.06a	136.67±7.37a	40.33±5.03a	53.00±4.58a
Powder 5%	98.33±4.93b	109.00±8.19b	44.33±5.69a	32.33±12.10b
Powder 10%	77.33±3.51c	94.67±10.26b	42.00±4.58a	16.67±6.51cd
Extract 250	95.00±6.6b	106.33±17.95b	49.00±4.58a	24.67±1.53bc
Extract 500	67.33±7.51cd	74.67±4.04c	43.33±6.66a	9.00±2.65de
LSD at 0.05	10.95	17.91	ns	10.77

Significant variations in the results (p<0.05) are shown by different superscripts in the same column (a, b, c, and d); LSD stands for least significant variations; SD: Standard deviation of means; ns= non-significant; CH: cholesterol; TG: triglycerides; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein.

As illustrated in Table (7), STZ diabetic rats fed on broad bean (Vicia faba L.) pods powder and methanolic extract had significant increases (p<0.05) in the serum levels of liver enzymes (ALT and AST) which recorded 48.33±3.51 and 209.00± 8.00 U/L, respectively whereas a significant decrease in serum albumin was observed (3.51 ± 0.14) g/dl when compared with the negative control which recorded 23.33±4.51 U/L for AIT, 83.33 ±5.51 U/L for AST and 4.41 ± 0.12 g/dl for Alb. The results showed that broad bean pod powder and its alcoholic extract significantly lowered (P<0.05) the ALT and AST serum levels and increased the serum level of albumin when given orally to STZ diabetic rats at different dosage levels. These effects were dose-dependent. The most effective concentration that caused the lowest decrease in liver enzymes was 500 mg/kg alcoholic extract of broad bean pods, followed by 10% broad bean pods powder in comparison to positive control rats at p<0.05.

According to data in Table (7), STZ diabetic rats (the positive control) had the highest significant (P<0.05) levels of uric acid and creatinine (1.07±0.08 and 2.97 ± 0.08) mg/dl, respectively as compared to the normal control which recorded 0.55 ±0.07 mg/dl and 1.65±0-03 mg/dl for the same parameters, respectively. Data revealed that oral administration of broad bean (*Vicia faba L.*) pods powder (5 and 10)% and broad bean pods alcoholic extract at doses (250 and 500) mg/kg to STZ diabetic rats for four weeks significantly (P<0.05) reduced the increased serum levels of creatinine and uric acid compared to the positive control

group, in a dose-dependent way. It was revealed that the most potent methanol extract in terms of antibacterial and antiradical properties was the one with the highest concentrations of total phenolic, flavonoid, and tannin. ALT, AST, alkaline phosphatase activity, urea, uric acid, and creatinine levels were lowered in blood in mice with alloxan-induced diabetes when a methanol extract (500 mg per kg body weight) was given orally to the animals [14]. All of the aforementioned indicators tended to return to normal following administration of the BBP extract, indicating both the drug's promise for preventing diabetes and its capacity to protect the liver and kidneys from damage brought on by STZ. Reducing free fatty acids and their peroxide as well as inhibiting oxidation, phosphorylation, and inflammation may be ways to restore ALT, AST, alkaline phosphatase, urea, uric acid, and creatinine levels [58]

Table 7: Influence of broad bean pods powder and extract on liver enzymes, creatinine and uric acid of STZ diabetic rats

Groups	ALT (U/L) Mean±SD	AST (U/L) Mean±SD	Alb (g/dl) Mean±SD	Creatinine (mg/dl) Mean±SD	Uric acid (mg/dl) Mean±SD
Control (-)	23.33±4.51d	83.33±5.51d	4.41±0.12a	0.55±0.07d	1.65±0.03e
Control (+)	48.33±3.51a	209.00±8.00a	3.51±0.14d	1.07±0.08a	2.97±0.08a
Powder 5%	47.67±2.08a	199.33±12.50a	3.35±0.22d	0.79±0.09b	2.96±0.14a
Powder 10%	32.00±2.0bc	117.33±15.01c	4.13±0.08b	0.63±0.04cd	2.07±0.11c
Extract 250	37.33±4.73b	178.33±7.51b	3.75±0.15c	0.71±0.07bc	2.50±0.08b
Extract 500	29.00±2.0cd	130.33±6.51c	4.20±0.05ab	0.59±0.08cd	1.87±0.04d
LSD at 0.05	5.96	16.14	0.24	0.13	0.16

Significant variations in the results ($p<0.05$) are shown by different superscripts in the same column (a, b, c, and d); LSD stands for least significant variations; SD: Standard deviation of means; ALT: alanine transaminase; AST: aspartate aminotransferase; Alb: serum albumin

3.3.5.Sensory evaluation of Falafel supplemented with broad bean pods paste "with and without seeds"

Table (8) illustrates the sensory evaluations of falafel made by 25 panelists.. Broad bean pods "with and without seeds" were added to the paste in three concentrations: 20, 30, and 40%. After testing a variety of doses during the pilot study conducted before the start of the research, these amounts were the most widely accepted. The addition of whole broad bean pod paste had no significant impact on the color, flavor, consistency, acceptance, or taste of the falafel in the control group or 20%. However, the addition of 30 and 40% of this paste notably ($P<0.05$) influenced the above parameters.

The addition of whole broad bean pod paste "with seeds" notably ($P<0.05$) affected the overall acceptance of falafel on its flavor, color, consistency, odor, and acceptability. According to the data in

Table 8, the sample containing 20% intact broad bean pods had a mean value for color that was significantly greater than the samples comprising 30% and 40% (8.80, 8.40, and 7.20) respectively. On the other hand, the control, 20, 30, and 40% samples' respective mean odor values were 9.20, 8.80, 8.40, and 8.20. These findings showed that the control and 20% samples had the highest mean consistency values, while the 30 and 40% samples had the lowest. It was discovered that adding whole broad bean pods at a rate of 20% enhanced the taste mean values. The taste mean values for the 30% and 40% samples were significantly different (8.60 and 7.80), respectively. The degree of overall acceptability increased when whole broad bean pods were added at a 20% rate compared to the control, 30 and 40% samples (8.40±0.89, 8.80±0.84 and 7.40±0.55) respectively.

Table (8) shows that using a 30% concentration of broad bean pod paste "without seeds" did not significantly improve the flavor of the falafel when compared to using a 40% concentration (7.40 and 6.60), respectively. The mean color values for the control, 20, 30, and 40% of the falafel, on the other hand, were 8. 20, 8.00, 8.40 and 7.80, respectively. These findings showed that the control and 30% of the falafel had the highest mean value of color, while 20% and 40% of the falafel had the lowest. The addition of a high amount of broad bean pod paste "without seeds" (40%) was observed to reduce odor mean values.

It can also be seen from Table (8) that no statistically significant ($P<0.05$) differences were seen in the average values of overall acceptance between the control falafel and the 30% samples. In comparison to other samples, the control falafel sample had the greatest mean consistency value (9.40±0.55), according to the findings. Furthermore, a 20% addition of broad bean pod paste "without seeds" raises overall acceptability (9.00±0.71) compared to falafel with 30% and 40% (8.60±0.55 and 7.80±0.84), respectively. The falafel with 20% broad bean pod paste "without seeds" performed the best in the sensory evaluation when compared to other concentrations, according to the data shown here.

The addition of broad bean pod fibers significantly enhanced the bread's texture profile, according to a bread evaluation [59]. Broad bean was shown to be a 35%-level substitution for kocho by Serka et al.,[60]. Additionally, it demonstrated that adding broad beans and kocho to bread increases its nutritional value without sacrificing its sensory appeal. Broad bean pod fiber can be utilized to improve bread's texture quality and dough development [59].

Table 8: The sensory evaluation of Falafel made of paste supplemented with broad bean pods "with and without seeds"

Whole green broad bean pods "with seeds"					
Treatments	Tasts Mean±SD	Color Mean±SD	consistency Mean±SD	Odor Mean±SD	Overall acceptability Mean±SD
Control	9.60±0.5a	8.20±0.84a	9.40±0.55a	9.20±0.84a	8.40±0.89b
20%	9.20±0.45ab	8.80±0.45a	9.00±0.71a	8.80±0.84a	9.40±0.55a
30%	8.60±0.55bc	8.40±0.55a	9.00±0.71a	8.40±0.89a	8.80±0.84ab
40%	7.80±0.84c	7.20±0.84b	7.80±0.84b	8.20±0.84a	7.40±0.55c
LSD at 0.05	0.82	ns	Ns	ns	0.97
Green broad bean pods "without seeds"					
Treatments	Taste Mean±SD	Color Mean±SD	Consistency Mean±SD	Odor Mean±SD	Overall acceptability Mean±SD
Control	9.60±0.55a	8.20±0.84a	9.40±0.55a	9.20±0.84a	8.40±0.89ab
20%	8.40±0.55b	8.00±0.71a	7.60±0.55b	8.00±0.71b	9.00±0.71a
30%	7.40±0.55c	8.40±0.55a	7.80±0.84b	7.00±0.71c	8.60±0.55ab
40%	6.60±1.14c	7.80±1.64a	8.00±1.00b	6.60±0.55c	7.80±0.84b
LSD at 0.05	0.99	ns	ns	0.94	1.02

Significant variations in the results ($p<0.05$) are shown by different superscripts in the same column (a, b, c, and d); LSD stands for least significant variations; SD: Standard deviation of means; ns= non-significant.

Microscopic pictures of H&E stained pancreatic sections show normal (**α** cells; narrow dashed arrows& **β** cells wide dashed arrows) of islets (i), exocrine pancreatic acini (e), pancreatic ducts, blood vessels and interstitial tissue in the control normal group (Photo 2). Pancreatic sections from STZ diabetic group show dilated pancreatic ducts (thick black arrows), congested blood vessels (red arrows), and leukocytic cells infiltration (black arrows). Other pancreatic sections from the STZ diabetic group show vacuolation in the exocrine acini (e) (arrowheads). In STZ diabetic group, widened interstitial space (*) accompanied with shrinkage in exocrine acini is also seen. slets showing necrotic **α** cells (narrow dashed arrows) with marked loss of **β** cells (red arrowheads). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50 (Photo 3).

Microscopic pictures of H&E stained pancreatic sections from the treated group received 5% broad bean pods powder showing slightly widened interstitial space (*) with mild shrinkage in exocrine acini, a few dilated pancreatic ducts (thick black arrows), few congested blood vessels (red arrows), and a few leukocytic cells infiltration (black arrows) in interstitial space between exocrine acini (e). Some islets in the treated group received 5% broad bean pods powder showing few necrotic **α** cells (narrow dashed arrows) with mild loss of **β** cells

(red arrowheads). Low magnification X: 100 bar 100 and high magnification X: 400 bar 50 (Photo 4). Pancreatic sections from treated group received 10% broad bean pods powder showing widened interstitial space (*), a few congested blood vessels (red arrows), mild loss of cells in islets (i) (red arrowheads) (Photo 5). Microscopic pictures of H&E stained pancreatic sections from treated group receiving 250 mg/kg broad bean pods extract showing perivascular leukocytic cells infiltration (black arrows) with normal cells of islets (i) and normal exocrine acini (e) (Photo 6). Pancreatic sections from the treated group receiving 500 mg/kg broad bean pods extract showed a retained normal picture of cells of islets (i), exocrine pancreatic acini (e), pancreatic ducts, blood vessels and interstitial tissue. Low magnification X: 100 bar 100 and high magnification X: 400 bar 50 (Photo 7).

These findings are consistent with those of Mejri et al. [14], who discovered that oral administration of broad bean pod (BBP) extracts to alloxan-induced diabetes mice led to a pronounced restoration of the islets of the acinar cell structure. These findings demonstrated that rather than having a direct impact on their functions, BBPs' anti-diabetic effects were related, at least in part, to their promotion of -cell growth and proliferation, which enhanced insulin production.

Given the biochemical traits, it would be logical to assume that the antioxidant and anti-inflammatory properties of the BBP extract, as well as its capacity to boost the pancreatic antioxidant defense, might have contributed to the pancreatic cells' regeneration and repair. An appealing approach to treating diabetes is the regeneration of pancreatic beta-cells by a natural antioxidant. The usefulness of several plant extracts and extracted antioxidant components in treating diabetes-induced oxidative damage in the pancreas was highlighted in earlier studies [61&62] in this regard.

The previous authors demonstrated that giving the natural antioxidant oleuropein to diabetic rats given alloxan effectively restored pancreatic beta-cell regeneration, reduced free radical production, and increased enzymatic and non-enzymatic antioxidants [61].

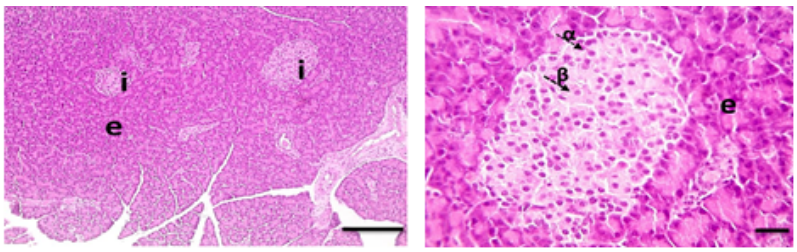

Photo 2: Normal control

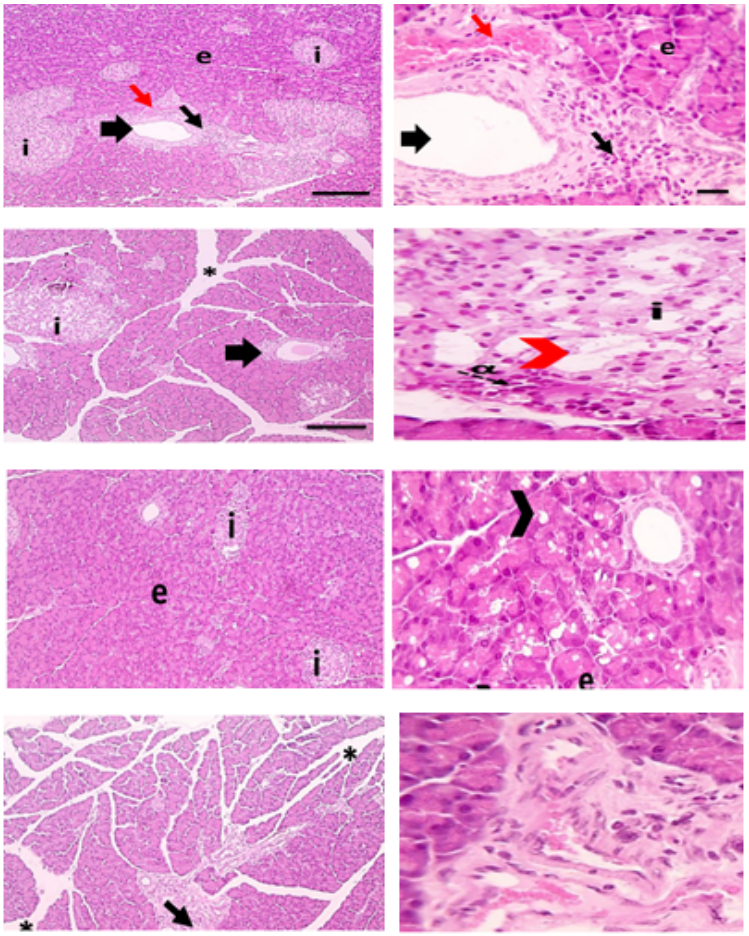

Photo 3: STZ diabetic group

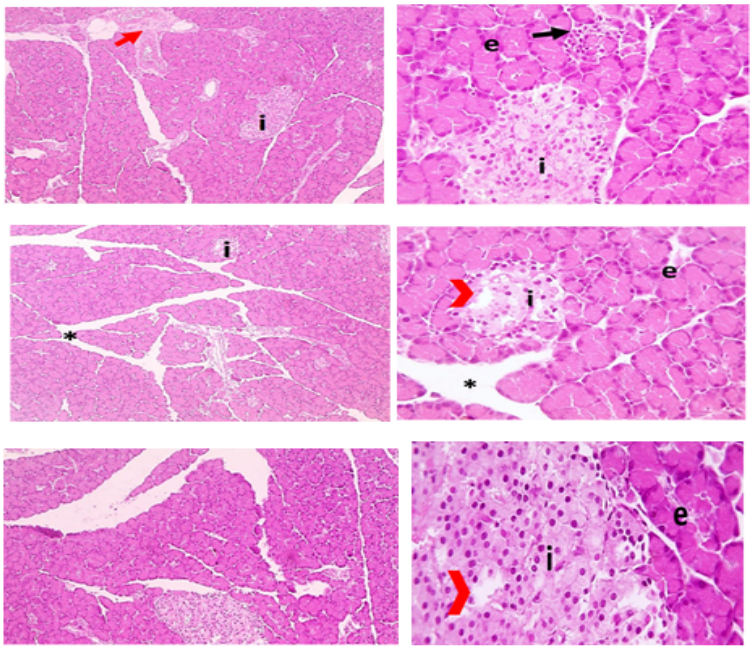

Photo 4: Treated group with powder 5% broad bean pods powder

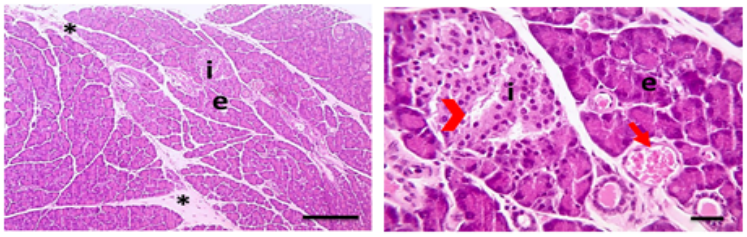

Photo 5: Treated group with powder 10% broad bean pods powder

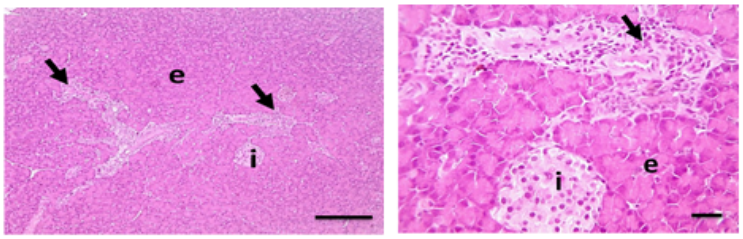

Photo 6: Treated group with 250 mg/kg broad bean pods extract

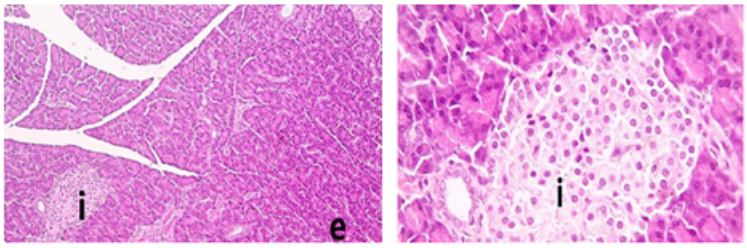

Photo 7: Treated group with extract 500 mg/kg broad bean pods extract

4- Conclusion

The findings of this study clearly show that broad bean pods, which are typically wasted, could be a valuable source of nutraceuticals such as carbs, fiber, proteins, and amino acids. Additional in vivo research validated the ability of the methanol extract to treat diabetic rats caused by streptozotocin. The data related to biochemical and histological investigations indicates that this activity is due to pancreas-protective effects of the test formulations. Based on this it can be suggested that the broad bean pods might make an alluring adjuvant in the treatment of diabetes and its consequences. The findings provide new opportunities for the responsible use of broad bean pod by-products and their valorization in food, feed, and medicinal applications. The organoleptic characteristics of the falafel were improved by the addition of the whole broad bean or with the waste pods only up to 20% so these results imply that the byproduct of broad bean pods may be a useful functional diet for the treatment of diabetes and its consequences.

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التغذية وعلوم الأطعمة

التقييم الغذائي والبيولوجي لمخلفات قرون الفول الأخضر في الفئران المصابة بداء السكري المستحث بالاستربتوزوتوسين

منى ياسر عبد الخالق مصطفى

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الملخص العربى:

الهدف من هذا البحث هو تقييم القيمة الغذائية وتأثير مخلفات قرون الفول الأخضر على نسبة السكر والدهون فى الدم لدى الفئران المصابة بداء السكري، بالإضافة إلى تأثيرها على وظائف الكبد والكلى، حيث تم تقسيم ثلاثين فأراً من ذكور سلالة ألبينو إلى ست مجموعات (5 لكل مجموعة) مجموعة منهم المجموعة الضابطة السالبة في حين باقي المجموعات الأخرى تم حقنها بمادة الاستربتوزوتوسين لإحداث مرض السكري. إحدى هذه المجموعات المصابة بالسكري تم اعتبارها كمجموعة ضابطة موجبة، وتم اعطاء باقى المجموعات المصابة بتركيزين من مسحوق مخلفات قرون الفول الأخضر (G3&G4) ومستخلص القرون (G5&G6)، تم إجراء التقييم الحسى على الفلافل المقلية المدعمة بالقرون الخضراء. وأظهرت النتائج أن المجموعات التى تم تغذيتها بمسحوق القرون (10%) والمستخلص الميثانولى بتركيز 250 و500 ملجم/كجم من وزن الجسم أظهرت انخفاضاً معنوياً فى نسبة الجلوكوز فى الدم، HbA1C، TC، TG، LDL-c بالإضافة إلى أنزيمات الكبد وظائف الكلى. وقد لوحظت زيادات كبيرة فى مستوى HDL-c فى مصل الدم وألبومين المصل فى المجموعات المعالجة مقارنة بالمجموعة الضابطة الموجبة .أظهر الفحص النسيجي تحسناً فى البنكرياس فى المجموعات المعالجة .أظهر التقييم الحسى للمنتج الشهير "الفلافل المقلية" أفضل النتائج مع العجينة المدعمة بنسبة 20%. أظهرت النتائج أن مخلفات قرون الفول الأخضر، التى يتم إهدارها عادة، يمكن أن تكون مصدرا قيما للمغذيات مثل الكربوهيدرات والألياف والبروتينات والأحماض الأمينية، كما أثبتت الأبحاث التى أجريت على الجسم قدرة مستخلص الميثانول على علاج الفئران المصابة بداء السكري الناجم عن الاستربتوزوتوسين. وبناءً على ذلك يمكن القول بأن مخلفات قرون الفول الأخضر قد تكون بمثابة مساعد مغري في علاج مرض السكري وعواقبه.

الكلمات الكاشفة: قرون الفول الأخضر، المخلفات، مضاد السكري، التشريح المرضي، الفلافل