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Abstract:

This study evaluated butterfly pea flower extract (BPFE) as a potential nutraceutical manage the complications of Metabolic Syndrome (MetS). The study conducted on 35 male albino rats weighing (170±10g) were divided randomly into two main groups. Group (I) n= (7) fed on a basal diet as the negative control. Group (II) n= (28) were fed a high-fat, high-carb diet (HFCD) before injected by Streptozotocin (STZ) at a dose of 35 mg/kg to create the MetS model. Then divided into four groups (7 rats each), one of them kept as a positive control group, the three left groups were given orally BPFE with doses of 100,200, and 300 mg/kg BW, respectively The study was assigned for 28 day. It was concluded that the extract of the BPF shows had a high content of protein, also antioxidant activity with anthocyanin, tannin and flavonoid content equal to (78.02, 1420.90 mg/100g and 66.78mgQ/g), respectively. Our nutritional and biological results imply that BPFE alleviates metabolic problems by lowering blood glucose levels, lipid profile and atherogenic index, while, enhanced insulin, HDL-c levels, liver enzymes levels. Furthermore, it was successful in restoring the status of oxidative stress (MDA, SOD, CAT, GSH) and inflammatory indicators, such as tumor necrosis factor (TNF-α). Histological examination of liver and pancreas tissue confirmed the results of biochemical analyzes of blood. So that the: It can be recommended that these Butterfly Pea Flower extract could be administered to Metabolic Syndrome when consuming high-fat high-carbohydrate diet.

Keywords: *Clitoria ternatea*, Metabolic diseases, Dyslipidemia, Diabetes mellitus, Antioxidant, Experimental animals.

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Introduction

In recent decades, metabolic syndrome (MetS) have become much more common worldwide. Statistics show that between 14% and 32% of people worldwide suffer from this ailment, and that both sexes are more likely to get it as they age **(Francini-Pesenti *et al.*, 2019)**. Obesity, insulin resistance, oxidative stress, and dyslipidemia, are among the disorders that make up the (MetS) **(Ashour *et al.*, 2023)**. At least three of the following five criteria must be met in order to be diagnosed with (MetS): Reduced HDL levels (<40 mg/dl), increased triglyceride levels (>150 mg/dl), obesity (body weight gain of ≥20%), hypertension (systolic blood pressure ≥130 mmHg and/or diastolic ≥85 mmHg), and hyperglycemia (RBG ≥200 mg/dl) **(Gunawan *et al.*, 2023)**. By reduced body weight and hyperglycemia can minimize the risk of (MetS) **(Mohamed *et al.*, 2023)**.

The first-line treatments for MetS include dietary and physical activity changes as well as lifestyle modifications. It has been shown that certain dietary components and natural compounds, referred to as nutraceuticals, can aid in the treatment of MetS **(Ahire *et al.*, 2023)**. Natural products have the advantages of great safety and few negative effects when compared to pharmaceutical medications. The extract from *Clitoria ternatea* L. (CTE), also known as the butterfly pea flower (BPFE), is one such product **(Mohamed *et al.*, 2024)**.

BPF is composed of bioactive compounds, mainly tannins, saponins, triterpenoids, flavonoids, phenols, flavonol glycosides, alkaloids, proteins, anthraquinones, and anthocyanins, which are known for their strong anti-inflammatory and antioxidant qualities **(Harefa *et al.*, 2024)**. Promising pharmacological qualities of the butterfly pea flower include liver-protective, anti-diabetic, and anti-dyslipidemic effects **(Widowati *et al.*, 2024)**. Also, **Maulidy *et al.*, (2022)** found that in obese rats fed a high-fat diet, BPFE could lower total cholesterol levels. Furthermore, studies have demonstrated the potential benefits of BPFE in the management of dyslipidemia and diabetes mellitus **(Widowati *et al.*, 2023)**. As a result, this study evaluated butterfly pea flower extract (BPFE) on metabolic syndrome

in rats injected by Streptozotocin (STZ) and fed high-fat high-carbohydrate diet (HFCD).

Materials and methods

Materials

Plant:(*Clitoria ternatea* L.) or butterfly pea petals flowers, were acquired from the National Research Center in Giza, Egypt.

Chemicals: Cellulose, Casein, vitamins, minerals were acquired from the General Company for Commerce and Chemicals in Cairo, Egypt. STZ (98.0% purity), Quercetin-3β-D-glucoside were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA).

Kits for determination of all different parameters were purchased from Sigma-Aldrich Corp., MO, for use in analysis.

Rats: Thirty Five male rats weighing (170±10 g) were purchased from the animal house of the National Research Center in Giza, Egypt. Hygienic conditions such as a 12-h light/12-h dark cycle, 45–55% relative humidity, and 23–25 ^{0}C RT with feed and water available ad libitum were kept.

Methods:

Preparation of Butterfly Pea flower aqueous extract

After being cleaned with distilled water, the petals were allowed to dry for two days, then combined and ground with rubber-edged mills. Five liters of distilled water were used to extract the 1 kilogram crushed material, which was then boiled for 30 min. The nylon was used to filter the soluble extract. After being freeze-dried, the BPFE was kept at -20°C for use in subsequent animal tests **(Zakaria *et al*., 2018)**.

Chemical investigations

Moisture, protein, fat, ash, and crude fiber were measured by Official Agricultural Chemists **A.O.A.C (2010)**. By using the differential, total carbohydrates were computed.

Determination of total anthocyanin, tannin, flavonoid contents and antioxidant activity

1 mL of BPFE was added to two sample solutions (KCl buffer with pH 1.0 and ammonia buffer with pH 4.5) until the volume reached 10 mL (dilution factor/DF = 10). A shift in color from pink-red to blue-violet indicates the presence of anthocyanin, whereas a purple-violet hue indicates the presence of tannins **(Le *et al.*, 2019)**. The sample's absorbance was then measured using a UV-Vis spectrophotometer set to wavelengths between 500 and 700 vis-max and 700 nm. The following formula is used to determine

Total Anthocyanin/Tannin = $\frac{\text{Absorbance} \times \text{DF} \times 1000}{55.9 \times 1}$

- The aluminum chloride colorimetric method was used to assess the total flavonoid levels in the BPF flower extract according to **Madaan *et al.*, (2011)**, using Quercetin as standard to create the calibration curve. The extract's flavonoid content was stated as mg/g of QG equivalent (QGE) of the extract.

- Following the addition of the diluted BPFE to a 5.0 mL volumetric flask, 1.0 mL of a 0.4 mM 1,1-diphenyl-2-picryl hydrazyl (DPPH) solution was added as a testing specimen, and a DPPH solution containing methanol was added as a control specimen. Afterwards incubated for 30 min at 37^{o}C **(Sasmana *et al.*, 2024a)**. The wavelength used to measure the absorption is 515 nm. The proportion of inhibition was then computed using the following formula:

Inhibition (%) = $\frac{\text{Control Absorbance} - \text{Testing Specimen Absorbance}}{\text{Control Absorbance}}$

Biological Experiment

Ethical approval: The study began after receiving approval from Port Said University Nursing Faculty Research Ethics Committee, code number: NUR (1-12-2024) (44).

After a week of acclimation, rats were divided randomly into two main groups. **Group (I):** (n =7) rats fed a basal diet according to **(Reeves *et al.*, 1993)**, which served as a negative control group. **Group (II):** (n = 28) were fed (HFCD) consisting of 45% fat (animal lipid), 15% protein, 5.5 % fiber, 4.5% vitamins and minerals mixture were prepared as described by **Reeves *et al.*, (1993)**, with a 30% fructose solution in their drinking water for 6 weeks. Rats fed HFCD were given a single intraperitoneal (i.p.) injection of a low dose of STZ (40 mg/kg bw) **(Huang, *et al.*, 2022)**. Rats were tested for successful induction of diabetes after 7 days of STZ injection. The study only included rats classified as having type 2 diabetes if their blood glucose level was ≥200 mg/dl.

Then MetS model by (HFCD/STZ) were re-divided into 4 equal groups as following:

- **Group (2):** MetS rats, served as the positive control group.
- **Group (3):** MetS rats were orally administrated with a low dose of BPFE (100 mg/kg BW, by gavages).
- **Group (4):** MetS rats were orally administrated with a medium dose of BPFE (200 mg/kg BW, by gavages).
- **Group (5):** MetS rats were orally administrated with a high dose of BPFE (300 mg/kg BW, by gavages). The second group of rats was maintained on HFCD for the remainder of the experiment.

Every week, body weights were recorded during the 28-day course of each treatment. Blood samples were obtained by retro-orbital sampling after the rats had been anesthetized. After the animal was slaughtered and cleaned with regular saline, the liver and pancreas tissues were quickly removed.

Biological Evaluation

The amount of food intake (FI) was recorded daily, while rat's weight was measured once a week to identify the body weight gain. In according to **Chapman *et al.*, (1959)**, body weight growth (BWG %) and feed efficiency ratio (FER) are calculated using the following equation:

$$\mathbf{BWG\%} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

FER= Body weight Gain (BWG) (g)/day

Feed intake (FI) (g)/day × Experiment period (day)

Determination of Biochemical analysis

Glucose: Serum glucose measured by enzymatic GOD / POD kits according to **Burrin and Price, (1985)**. The enzyme-linked immune sorbent assay (ELISA) method is used to quantify insulin, as explained by **(Clark and Hales, 1994)**.

Liver Function: According to **Bergmeyer *et al*., (1978)**, liver function was assessed by measuring aspartate aminotransferase (AST) and alanine aminotransferase (ALT). **Belfield and Goldberg, (1971)** were used to assay alkaline phosphatase (ALP).

Lipid profile: Triglycerides (TG), total cholesterol (TC), and cholesterol contents of high density lipoprotein (HDL-c) were measured in accordance with **Fossati and Prenape, (1982)**, **Allian *et al.* (1974)** and **Albers *et al*., (1983)**, respectively. **Fruchart, (1982)** equation is used to calculate low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c).

LDL-c = TC-[HDL-c + (TG/5)] VLDL-c = TG/5.

This atherogenic index was calculated as the method described by **Sasmana *et al*., (2024a)**

Atherogenic Index = Total Cholesterol – HDL-C

HDL-C

Oxidative and Antioxidant Biomarkers: For assessing lipid peroxidation, the plasma level of Malondialdehyde (MDA) was identified by **Draper and Hadley, (1990)**. Superoxide dismutase (SOD) was assessed according to **Spitz and Oberley, (1989)**. Catalase (CAT) and reduced glutathione (GSH) levels were measured methods by **Aebi, (1984)** and **Habig *et al*., (1974)**, respectively.

Inflammation Markers: Pro-inflammatory cytokine tumor necrotic factor-α (TNF-α) was assessed using kits that were sold commercially in accordance the instructions of the manufacturer (Ray Biotech, Inc., Norcross, GA) **(Kandir and Keskin, 2016)**.

Histopathological examination:

The fixed samples of Liver and pancreatic in 10 % neutral buffered formalin were cleared in xylol and embedded in paraffin 3-5 µm thick section and stained with Hematoxylin and Eosin (H&E) for subsequent histopathological examination **Pashapoor *et al*., (2020)**.

Statistical analysis

The data were presented as mean ±SE, and a post hoc (Tukey's) test was used to analyze the mean difference after a one-way ANOVA. At ($P \leq 0.05$), the difference in values was deemed statistically significant **(Armitage and Berry, 1987)**.

Results and Discussion

Table (1) indicated the results of the proximate analysis in the butterfly pea flower including moisture 10.14±0.35, protein 45.83±1.26, ash 11.78 ±1.03, fat 2.65 ±0.54, crude fiber 3.76 ± 0.57, and carbohydrate 25.84 ±0.05. The chemical composition of the BPE was nearly identical to that of the earlier investigation by **Kovitvadhi *et al*., (2024)** observed that the amounts of crude protein (CP), ash, crude fiber, and starch in BPE are 43.7%, 11.4%, 2.90, and 23.4%, respectively. Similar, **Muhammad Ezzudin and Rabeta (2018)** who found that higher protein content in the leaves and flower of *C. ternatea*. In addition, **Umami *et al*., (2022)** showed that BPF without extraction to contain protein, ash, and crude fiber in amounts ranging from (25.11 - 28.62%), (6.71 - 8.36%), and (15.78 - 19.66%), respectively. The differences in results of the proximate analysis in BPFE due to contributed to the extraction methods.

Table (2) indicated the results of extraction of anthocyanin, tannin, total flavonoid content and antioxidant activity of from BPF have been reported to be (78.02 , 1420.90 mg/100 g, 66.78mg Q/g and 193.50 µg/mL), respectively. This result is agreement with **Sasmana *et al*., (2024)** observed

that total anthocyanin, tannin content and antioxidant activity of CTE 78.0943, 1424.90 mg/100 g and 194.26 µg/mL respectively. BPF has demonstrated substantial DPPH radical scavenging action ،due to it has a high anthocyanin content **(Gollen *et al*., 2018)**. Furthermore, **Utami *et al*., (2024)** observed that flavonoids were present in 68.003 mg QE/g of the *C. ternatea* ethanol extract and 78.767 mg QE/g of the ethyl acetate fraction.

Table (1): Proximate analysis of (BPFE)

Nutrients	Butterfly Pea flower extract (BPE) %
Moisture	10.14 ±0.35
Protein	45.83 ±1.26
Ash	11.78 ±1.03
Fat	2.65 ±0.54
Crude fiber	3.76 ± 0.57
Carbohydrate	25.84 ±0.05

*The results are the mean of three different determinations ± standard Error.

Table (2): Total anthocyanin, Tannin, Flavonoid content and Antioxidant activity of (BPFE)

Sample / Parameters	PBFE
Anthocyanin content	78.02 mg/100 g
Tannin content	1420.90 mg/100 g
Total Flavonoid content	66.78 mg/Q*
Antioxidant activity** (DPPH %)	193.50 µg/mL

* Quercetin equivalents / g of dry extracts

** Expressed as Ascorbic acid equivalent

Data in **Table (3)** showed that the control positive rat group which injured of Metabolic Syndrome showed a significant increase in final weight, weight gain, weight gain %, food intake and food efficiency ratio ($P \leq 0.05$) compared with negative control group. No significant variations in IBW of the all groups. This is consistent with **Mohamed *et al*., (2024)** showed that using a (HFCD) with an injection of STZ, the symptoms and indications of the metabolic syndrome have been imitated in rats. While, the

rat groups which injured of Metabolic Syndrome with BPFE at levels 100, 200 and 300 mg/kg BW showed a significant decrease ($P< 0.05$) in weight gain percent, food intake and FER compared with positive control group. Due to it contains substances like triterpenoids, tannins, saponins, and flavonoids are effective for weight loss. This is consistent with **Sasmana *et al*., (2024a); Haryati *et al*., (2024)** showed that BPFE may help obese rats lose weight. Similarly, **Utami *et al*., (2023)** showed that administration of BPF has beneficial effects on the reduction of BW in rats with HFD. Also, **Wang *et al*., (2022)** showed the aqueous of BPFE significantly prevented weight gain in mice with high fat high fructose diet (HFFD).

Table (3): Nutritional indicators of negative control and Metabolic Syndrome rat groups treated with Butterfly Pea Flower Extract (BPFE)

Parameters / Groups	IBW (g)	FBW (g)	BWG %	FI (g/day)	FER
Control (-ve)	169.67± 1.46^{a}	211.31 ±1.73^{c}	24.54±0.18^{d}	21	0.071±0.001^{d}
Control (+ve) HFCD/STZ	177.51 ± 0.89^{a}	301.12±2.16^{a}	69.64±1.43^{a}	29	0.152±0.004^{a}
HFCD+BPFE (Low)	170.73 ± 1.18^{a}	248.20±1.88^{b}	45.38±0.59^{b}	25	0.111±0.002^{b}
HFCD+BPFE(Medium)	173.85 ± 1.03^{a}	236.71±1.41^{b}	36.16±0.37^{c}	23	0.096±0.003^{c}
HFCD+BPFE (High)	175.94 ± 1.20^{a}	222.14±2.05^{c}	26.26±0.71^{d}	22	0.075±0.003^{d}

Initial body weight (IBW), Final body weight (FBW), Body weight gain (BWG%), food intake (FI) and feed efficiency ratio (FER). Results are expressed as mean ± SE .

Values in each column which have different letters are significantly different at ($P\leq0.05$).

Effect of BPFE toward serum glucose level and insulin in (MetS) rats are presented in **Table (4)**. FBG levels were within the usual range of <200 mg/dl in (-ve) control group. While, MetS model were made by HFCD/ STZ, causing hyperglycemia with FBG levels exceeding ≥200 mg/dl, resulting in an insulin deficiency, making it a valuable model for studying DM or MetS in line with other research **(Kiran *et al*., 2024; Widowati *et al*., 2024a)**. On the other hand, BPFE at different doses had an impact on lowering blood glucose and increasing insulin levels in metabolic

syndrome rats. Rat's blood glucose levels decrease in direct proportion to the amount of BPFE administered. This results consistent with **Sasmana *et al*., (2024a); Kiran *et al*., (2024)** observed that BPFE reduces glucose, enhances serum insulin levels of diabetic and dyslipidemia rats. Also, **Utami *et al*., (2024)** observed administered at 300 mg/kg BW of *C. ternatea* reduced in blood glucose levels. In line with previous study **Gunawan *et al*., (2023)** showed that rats with MetS can have their blood sugar levels lowered by BPE at doses of 100, 200, and 400 mg/kg. The many components of the BPF have pharmacological effects that may result in anti-hyperglycemia **(Sasmana *et al*., 2024)**.

Table (4): Blood glucose and insulin levels in control negative and Metabolic Syndrome rat groups treated with different levels of Butterfly Pea Flower Extract (BPFE)

Parameters / Groups	Fasting Blood Glucose (FBG) (mg\dl)	serum Insulin (µU/\ml)
Control (-ve)	90.38±2.82^{e}	3.22± 0.38^{a}
Control (+ve) HFCD/STZ	278.12±3.85^{a}	0.97 ± 0.23^{e}
HFCD+BPFE (Low)	155.90±2.04^{b}	1.86±0.27^{d}
HFCD+BPFE(Medium)	130.10±4.73^{c}	2.49±0.09^{c}
HFCD+BPFE (High)	110.06±3.64^{d}	2.93±0.16^{b}

Results are expressed as mean ± SE .

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Effect of BPFE toward on liver function are presented in **Table (5**). The HFCD/STZ raised the liver enzymes (AST, ALT, and ALP) activities in (MetS) rats compared to the negative control. One of the most important aspects of metabolic diseases is impaired liver function. This results agreement with **Nimrouzi *et al*., (2020)** reported that overconsumption of foods high in calories alters the liver natural function by exposing it to dense foods. In addition, liver, which stores glycogen, is one of the organs whose health is associated with diabetes mellitus **(Martuza *et al*., 2022)**. In

contrast, results showed that BPFE effectively reduced the effects of HFCD by considerably lowering the levels of the liver enzymes. The high dose, at 300 mg/kg BW, had a better effect than the low dose, but there was no discernible difference between the high and medium doses of BPFE. This is consistent with **Widowati *et al.*, (2024a)** showed that BPE can lessen liver damage by dramatically lowering ALP enzyme levels. Similarly, **Balaji *et al.*, (2015)** observed that rats given 500 mg/kg of BPE were decreased the liver enzyme levels. BPF has several flavonoid and phenolic components that improve the liver's ability to regenerate itself, which explains its hepatoprotective action **(Wan and Jiang, 2018)**.

Table (5): Effect of Butterfly Pea Flower Extract (BPFE) on AST, ALT and ALP of control negative and Metabolic Syndrome rat groups

Parameters / Groups	AST	ALT	ALP
	(µ/L)		
Control (-ve)	42.57±2.18^{d}	27.88±1.63^{e}	102.40±1.47^{d}
Control (+ve) HFCD/STZ	85.09±1.30^{a}	69.70±2.05^{a}	229.70±2.55^{a}
HFCD+BPFE (Low)	54.65±2.72^{b}	44.65±1.52^{b}	162.86±1.08^{b}
HFCD+BPFE(Medium)	47.82±1.13^{c}	35.72±1.34^{c}	134.73±1.39^{c}
HFCD+BPFE (High)	44.72±1.40^{cd}	34.91±0.90^{c}	128.29±1.62^{c}

Aspartate transaminase (AST); Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Results are expressed as mean ± SE .

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Effect of BPFE toward on lipid profile and atherogenic index in (MetS) rats are presented in **Table (6)**. When HFCD is given, the levels of TC, TG, LDL-c, VLDL-c, and AI increase while those of HDL-c decrease in comparison to the negative control. HFCD also caused value of TC to increase to >110 mg/dl, HDL levels to decline to <40 mg/dl, which, the rats meet the criteria for metabolic syndrome according to **Gunawan *et al.*, (2023)**.

In contrast, treatment of BPFE caused improving lipid profile, and increasing HDL. It was ascribed to the plant's flavonoid and polyphenol

content. This is corroborated by the study of **Haryati *et al*., (2024); Widowati *et al*., (2024)** found that BPFE at 400mg and 600 mg/kg BW lowers cholesterol in obese rats. **Sasmana *et al*., (2024a)** showed that BPFE supplementation reduce VLDL levels. This is explained by the fact that cholesterol binds to bile acids in the colon, causing the body to excrete cholesterol and prevent its reabsorption. In line with previous studies, **Utami *et al*., (2023)** showed that administration of BPF has beneficial effects on the reduction of lipid profile and the increasing of HDL-C levels in mice / rats with HFFD or HFD. Similar, **Arifah *et al*., (2022)** observed that administrations of 2% of the aqueous of BPFE for 15 weeks reduced lipid profile and increased HDL-c. Furthermore, this study found that the administration of BPFE resulted in a drop in the atherogenic index. **Harefa *et al*., (2024a)** showed that butterfly pea and Roselle combination extracts can to lower atherosclerosis biomarkers at a dose of 500 mg/kg BW. Also, **Sasmana *et al*., (2024a)** found that a connection between a reduction in the atherogenic index and anthocyanin in BPF supplementation. These compounds in BPF such as anthocyanins, tannins, glycosides, flavonoids, steroids, saponins, and phenols can lower cholesterol synthesis by blocking the enzyme HMG-CoA reduce **(Hakam -Maulidy *et al*., 2022)**.

Table (6): Effect of Butterfly Pea Flower Extract (BPFE) on Lipid Profile and atherogenic index of negative control and Metabolic Syndrome rat groups

Parameters / Groups	TC	TG	HDL-c	LDL-c	VLDL-c	AI
	(mg/dl)					
Control (-ve)	134.68±3.60^{d}	118.60±1.79^{d}	62.61±1.83^{a}	48.35±1.41^{e}	23.72±0.36^{c}	1.15±0.97^{e}
Control (+ve) HFCD/STZ	201.60±4.40^{a}	168.51±2.78^{a}	30.93±2.67^{e}	136.97±1.17^{a}	33.70±0.56^{a}	5.52±0.65^{a}
HFCD+BPFE (Low)	170.80±3.76^{b}	134.82±4.03^{b}	42.39±1.93^{d}	101.45±1.02^{b}	26.96±0.81^{b}	3.03±0.95^{b}
HFCD+BPFE (Medium)	152.34±3.90^{c}	126.27±3.61^{c}	48.73±1.64^{c}	78.36±1.54^{c}	25.25±0.72^{b}	2.13±1.38^{c}
HFCD+BPFE (High)	140.54±4.46^{d}	122.65±2.90^{cd}	54.33±2.18^{b}	61.68±1.70^{d}	24.53±0.58^{bc}	1.59±1.05^{d}

Total cholesterol (TC), Triglycerides (TG), High density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C). atherogenic index (AI). Results are expressed as mean ± SE .

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Data for Data for effect of BPFE on oxidative stress, and (TNF-α) in (MetS) rats are presented in **Table (7)**. The HFCD /STZ induction raised (MDA) concentration in liver tissue and (TNF-α), while the reduced antioxidant enzymes such as (SOD, CAT, GSH) concentration compared to the negative control group. This is consistent with **Priyanto *et al*., (2023)** state that hyperglycemia can increase oxidative stress markers such as (MDA) due to increased non-enzymatic glycosylation processes in proteins due to the oxidation of aldehyde groups in highly reactive glucose. Furthermore, **Li *et al*., (2023)** demonstrated that connections between diabetes, dyslipidemia, and inflammatory cytokines, specifically TNF-α.

In contrast, the findings of this study demonstrated that BPEF ingestion could considerably lower MDA concentration and key inflammatory cytokines (TNF-α) and raise SOD, CAT, and GSH, potentially halting the development and progression of metabolic diseases. BPFE at 300mg/kg BW was significantly more effective than its effect at a low dose. These results consistent with **Widowati *et al*., (2024a)** found that BPE increased SOD and CAT levels while decreasing MDA levels in diabetes mellitus and dyslipidemia rats. Similarly, **Utami *et al*., (2024);Putri *et al*., (2023)** observed that administration of BPF extract 300 and 600mg/Kg BW for 21 days can protect the body from oxidative stress linked to diabetes by significantly increasing the activity of antioxidant defense enzymes while lowering MDA levels. Furthermore, **Widowati *et al*., (2024)** observed that the butterfly pea extract at 800 mg/kg BW also reduces cytokines (TNF-α) in diabetic rats' liver Similarly, **Maneesai *et al*., (2021)** observed that BPF reduce the rate of (TNF-α). According to **Widowati *et al*., (2023**) reported that BPFE include a number of

pharmacological components have antioxidant properties that prevent oxidative stress-induced cell damage, and reduce inflammation.

Table (7): Effect of Butterfly Pea Flower Extract (BPFE) on oxidative stress, antioxidant enzymes and (TNF-α) of negative control and Metabolic Syndrome rat groups

Parameters / Groups	MDA (nmol/mg)	SOD (U/ml)	CAT (U/ml)	GSH (U/ml)	TNF-α (pg/ml)
Control (-ve)	11.54±0.62^{e}	17.53±1.06^{a}	10.85±0.32^{a}	9.89 ± 1.29^{a}	90.22±0.54^{e}
Control (+ve) HFCD/STZ	33.87±0.73^{a}	9.86±0.52^{e}	5.10±0.19^{d}	4.36 ± 2.33^{d}	300.54± 0.47^{a}
HFCD+BPFE (Low)	21.61±0.34^{b}	12.02±0.19^{d}	8.38±0.30^{c}	6.73 ± 1.10^{c}	204.08± 0.38^{b}
HFCD+BPFE (Medium)	15.07±0.29^{c}	14.63±0.73^{b}	9.66±0.61^{b}	7.84 ± 3.10^{c}	142.30± 0.43^{c}
HFCD+BPFE (High)	13.80±0.87^{d}	15.33±0.87^{b}	9.87±0.27^{ab}	8.60 ± 2.28^{b}	125.52± 0.50^{d}

Malondialdehyde (MDA), Superoxide dismutase (SOD); Catalase (CAT) Reduced glutathione (GSH) and Tumor necrotic factor-α (TNF-α). Results are expressed as mean ± SE .

Values in each column which have different letters are significantly different at ($P \leq 0.05$).

Histopathology examination

Under a microscope, the livers of the positive and negative control groups displayed different histological structures, including the normal hepatic lobule and portal vein, **(Photo1. A)**, hyperplasia of the bile duct's epithelial lining and fibrosis of its wall, **(Photo1. B)**, congestion of the central vein and hepatic sinusoids **(photo1. B1)**, and activation of Kupffer cells and apoptosis of hepatocytes **(photo1. B2)**. These results consistent with **Widowati *et al.*, (2024)** observed that STZ induction results in lipid degradation of the liver and pancreas in addition to inflammation.

The central vein and hepatic sinusoids of obese diabetic rats were enlarged in the liver tissues after it was fed 100 mg/kg BW/day of Butterfly Pea flowers extract (BPE) orally **(photo1. C)**, moreover, **(photo1. C1)** showed the liver tissues activation of Kupfer cells. Conversely, the rats in the oral BPE (200 mg/kg BW/day) showed mild congestion of hepatic sinusoids with hepatocyte binucleation **(photo1. D)**, while, the liver tissues of the rat appeared to have a normal histological structure **(photo1. D1)**. Conversely, liver sections from obese diabetic rats given oral doses of BPE (300 mg/kg BW/day) did not exhibit any histological changes **(photo1. E and E1)**.

This is consistent with **Widowati *et al*., (2024)** observed that BPE improved liver histopathology in DM and dyslipidemia rats. Also, **Khatib and Arisanty, (2024)** found *C. ternatea* dosages of 500 and 1000 mg/kg BW are safe, since no mice perished after receiving treatment for 14 days. These effects can be ascribed to the antioxidant properties of flavonoids and anthocyanin, which can combat free radicals, shield cells and tissues from harm, and prevent liver damage **(Utami *et al*., 2024)**.

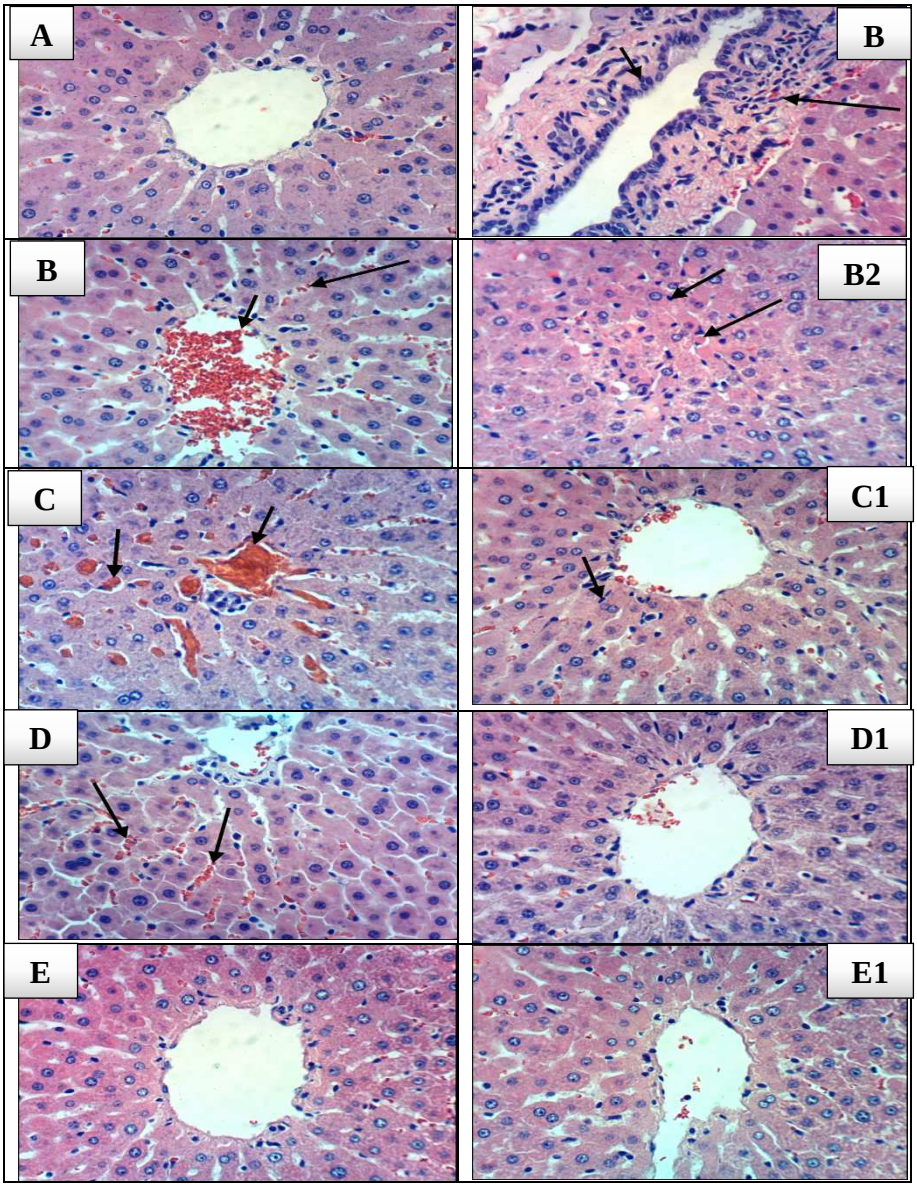

Photo (1): Photomicrograph of rats Liver (H&E X 400). A= groups (1) (ve-), showing the normal histological structure; B, B1 and B2=group(2) obese diabetic HFCD/STZ;C and C1= group (3) BPE 100 mg/kg; D and D1= group (4) BPE 200mg/kg and E and E1= group (5) group BPE 300 mg/kg.

Pancreas:

Normal control rats' pancreatic islet cells displayed no histopathological alterations in their histological appearance **(Photo2. A)**. Microscopic analysis of the pancreatic sections from the obese diabetic untreated group showed vacuolation of the epithelium lining the pancreatic acini **(Photo2. B)**, as well as showed necrosis and vacuolation of the islets of Langerhans cells **(Photo2. B1)**. Nonetheless, pancreatic sections of obese diabetic rats administered 100 mg/kg BW/day of BPE exhibited mild vacuolation of sporadic islets of Langerhans cells **(Photo2. C)**. Conversely, pancreatic islet tissues from obese diabetic rats that received an oral dose of 200 mg/kg BW/day of BPE displayed a small amount of vacuolation in sporadic islets of Langerhans cells **(Photo2. D)**, while pancreatic tissues of the rat did not exhibit any histological alterations **(Photo2. D1)**.After oral administration of 300 mg/kg BW/day of BPE to obese diabetic rats, no histological alterations were observed in the pancreatic sections studied **(Photo2. E and E1)**. This finding was corroborated by earlier research showing that the flavonoid chemicals in *C. ternatea* can shield pancreatic cells from oxidative stress-induced oxidative damage, hence preserving the cells' ability to produce insulin normally **(Fani-Temarwut *et al*., 2023)**.

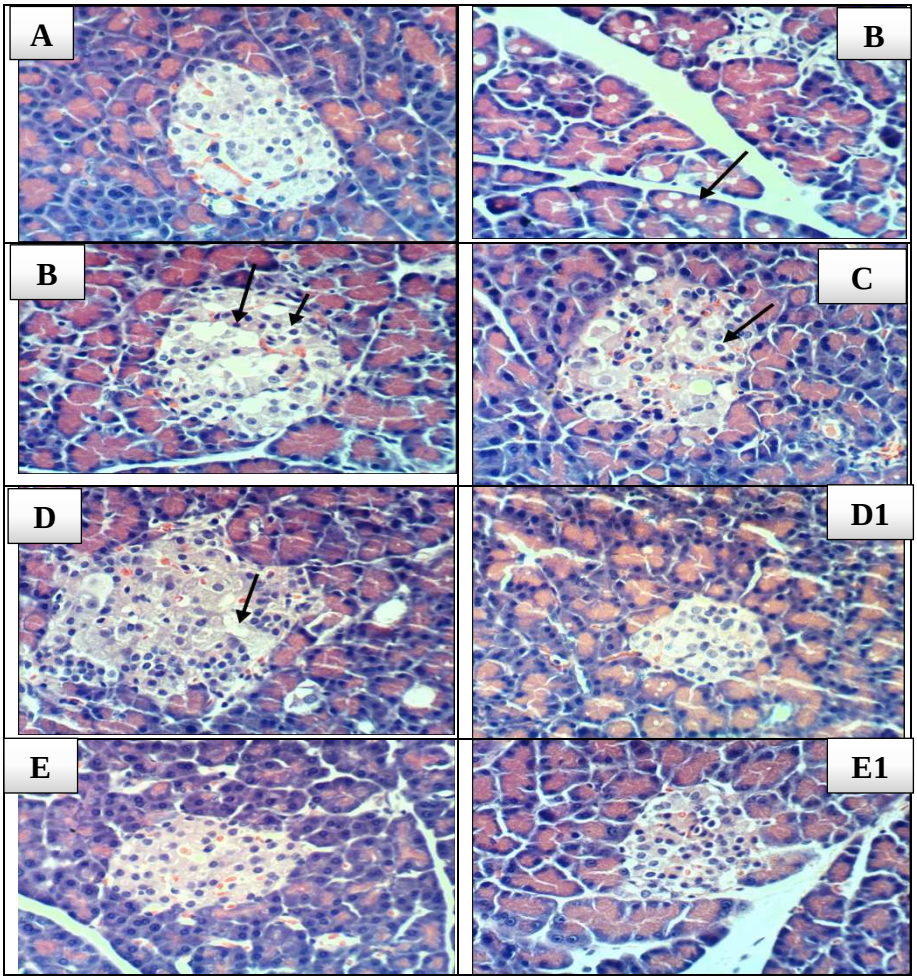

Photo (2): Photomicrograph of rats Pancreas (H&E X 400). A= groups (1) (ve-), showing the normal histological structure;B and B1= group(2)obese diabetic HFCD/STZ ;C = group (3) BPE 100 mg/kg; D and D1= group (4) BPE 200mg/kg and E and E1= group (5) group BPE 300 mg/kg.

Conclusion

In conclusion, we recommend including butterfly pea extract (*Clitoria ternatea*) in diets for metabolic syndrome and hyperlipidemic complications because they have the antioxidant and anti-inflammatory

properties which ability to reduce body weight gain , MDA and cytokines (TNF-α) , blood glucose levels and lipid profiles and raises insulin levels, serum SOD, CAT, GSH levels, This suggests it is anti-metabolic syndrome and anti-diabetic effects.

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التأثير المحتمل لمستخلص زهرة بازلاء الفراشة (الشاى الأزرق) على متلازمة التمثيل الغذائي في الفئران التي تتغذى على نظام غذائي عالي الدهون والكربوهيدرات

شيماء حسن نجم * نجلاء فتحى محمد سالم *

الملخص العربي:

تمت هذه الدراسة علي مستخلص زهرة بازلاء الفراشة كغذاء علاجي مُحتمل لعلاج مُضاعفات متلازمة التمثيل الغذائي. أُجريت الدراسة على ٣٥ من ذكور الفئران البيضاء، بوزن (١٧٠ ± ١٠ جرام)، وقُسِّمت عشوائيًا إلى مجموعتين رئيسيتين. المجموعة الأولى (عددها ٧ فئران) تغذت على نظام غذائي قياسي كمجموعة ضابطة سالبة . والمجموعة الثانية (عددها ٢٨ فار) تغذت على نظام غذائي غني بالدهون والكربوهيدرات وذلك قبل حقنها بالستربتوزوتوسين (STZ) بجرعة ٣٥ ملجم/كجم للاصابة بمتلازمة التمثيل الغذائي. ثم تم تقسيمها إلى أربع مجموعات (٧ فئران لكل منها) حيث احتفظت إحداهما كمجموعة ضابطة موجبة وأعطيت المجموعات الثلاث الاخري عن طريق الفم مستخلص زهرة البازلاء الفراشية بجرعات ١٠٠ و٢٠٠ و٣٠٠ مجم/ كجم من وزن الجسم على التوالي. واستمرت الدراسة لمدة ٢٨ يومًا وخلصت نتائج الدراسة الي أن مستخلص زهرة بازلاء الفراشة يحتوي على نسبة عالية من البروتين، كما أظهر نشاطًا مضادًا للأكسدة مع محتوى الأنثوسيانين والتانين والفلافونويد (٧٨,٠٢، ١٤٢٠,٩٠ مجم/ ١٠٠ جم ،٦٦,٧٨ مجم / جم) على التوالي. وتشير النتائج الغذائية والبيولوجية إلى أن مستخلص زهرة بازلاء الفراشة يخفف من المشاكل الأيضية عن طريق خفض مستويات جلوكوز ودهون الدم ومؤشر تصلب الشرايين، بينما يزيد من مستوي الأنسولين والليبوبروتينات عالية الكثافة ويحسن مستويات إنزيمات الكبد. علاوة على ذلك القضاء علي حالة الإجهاد التأكسدي (من خلال خفض مستويات المانولدهيد MDA ورفع مستويات الانزيمات المضادة للاكسدة (SOD وCAT وGSH) وخفض المؤشرات الالتهابية، مثل عامل نخر الورم (TNF-α). وأكد الفحص النسيجي لأنسجة الكبد والبنكرياس نتائج التحاليل الكيميائية الحيوية للدم. لذا يُعد مستخلص زهرة بازلاء الفراشة علاج فعال لمتلازمة التمثيل الغذائي نظرًا لخصائصه المضادة للالتهابات ومضادات الأكسدة. وتوصي الدراسة باستخدام مستخلص زهرة بازلاء الفراشة لعلاج متلازمة التمثيل الغذائي ، مرض السكر الناتج من اتباع نظام غذائي غني بالدهون والكربوهيدرات في الفئران .

الكلمات المفتاحية : الشاى الازرق – أمراض التمثيل الغذائى – خلل دهون الدم - مرض السكر – مضادات الأكسدة- فئران التجارب .

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