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Egyptian "Ful Medames" Mitigates Metabolic Syndrome-Associated Inflammation through Regulation of Resistin, Leptin and Adipokine Mrna Expression in Wistar Rats

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In Loving Memory of Late Professor Doctor ""Mohamed Refaat Hussein Mahran""

Abstract

Metabolic syndrome is among the serious global health problems and is usually associated with inflammation. Dietary aspects contribution and genetic associations to minimize metabolic syndrome disorders and inflammation is a controversy point. The present study aims to evaluate the ability of the most popular Egyptian meals "Ful-Medames" to mitigate the metabolic syndrome associated disorders and inflammation in Wistar rats and its impact on the expression regulation of the white adipose tissue mediators; resistin, leptin and adiponectin. Three groups of male albino rats were used; negative, positive with induced metabolic syndrome and Ful-Medames fed group pre-induced for metabolic syndrome lasting for four weeks. Results revealed a high protein content of 26.56% for Ful-Medames, considerable antioxidant activity and polyphenolic compounds as detected by HPLC. Biochemical findings revealed restoring of the changed levels of blood glucose, total cholesterol, triglycerides, HDL-C, LDL-C, transaminases' activity, insulin resistance, CRP and hepatosomatic index with marked improvement of changes in histopathological and resistin, leptin and adiponectin expression for the Ful-Medames fed metabolic syndrome group. Thus, it can be concluded that "Ful-Medames" counteracts metabolic syndrome-associated disorders and inflammation through restoring the disordered expression regulation of resistin, leptin and adiponectin.

Keywords: Metabolic syndrome, inflammation, Ful Medames, resistin, leptin, adiponectin.

1. Introduction

Metabolic syndrome (MetS) is a term referring to a group of risk factors threatening public health and substituting a global clinical challenge. Its prevalence is rapidly increasing in the past few decades due to acquired wrong life styles reaching up to 10% - 30% in both developing and developed nations [1]. It is a cluster of metabolic disorders comprising hyperglycemia, atherogenic dyslipidemia, insulin resistance, high blood pressure and central obesity [1, 2] and it is characterized by prothrombotic and proinflammatory states [3]. According to the latest consensus of the American Heart Association, National Heart, Lung and Blood Institute and International Diabetes Federation, metabolic syndrome is defined if three of the following five criteria are satisfied; fasting blood glucose more than 100 mg/dl, increased triglyceride levels more than 150 mg/dl, reduced high-density lipoprotein cholesterol (HDL-C) levels less than 40 mg/dl, blood pressure more than 130/85 mmHg and increased waist circumference [4]. This collection of pathological and abnormal conditions is considered as a crucial risk factor for developing many diseases, like diabetes mellitus, cardiovascular diseases, chronic kidney disease and hypertension [5].

Although life style is the main determinant for metabolic syndrome development, yet genetic factors play an important role [6].

Nowadays, light was thrown on the crucial role of the adipokines that are secreted by white adipose tissue. The latter is considered as an active endocrine

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organ that is capable of secreting some regulatory mediators which are adipokines. These adipokines are said to be involved in many biological processes, among which inflammation, oxidative stress, lipid metabolism and adipogenesis [2]. Up or downregulation of the protein expression of the genes responsible for the production of the three adipokines; resistin, leptin and adiponectin are strongly correlated with metabolic syndrome [2, 7, 8]. Resistin regulates insulin action, lipid, glucose and energy homeostasis [2] and its elevation is responsible for impaired insulin action and blood glucose [9] and it may serve as a molecular link between metabolic parameters, inflammation and vascular dysfunction participating to the risk for metabolic syndrome, cardiovascular diseases and type 2 diabetes mellitus [2, 10]. Adiponectin has many functions comprising anti-inflammatory, antiapoptotic, anti-fibrotic, antioxidant effects, controlling insulin and glucose, protecting against cardiovascular, colon, lung and heart diseases [8]. Leptin, which is the first discovered adipocytokine, is mainly responsible for insulin resistance, since it was found elevated in the serum of obese subjects [11], participating in the normal regulation of energy expenditure, body weight, food intake and neuroendocrine functions and involved in inflammation associated with metabolic syndrome and atherosclerosis [7].

Metabolic syndrome is reported to be linked with a chronic low-grade inflammation state [12] and those patients who are diagnosed with metabolic syndrome were found to have a state of activated inflammation [13]. Kir et al. [14] reported that many inflammatory markers were detected in patients with metabolic syndrome confirming that c-reactive protein (CRP) is still among the most reliable inflammatory markers in those patients.

There is no approved therapy for metabolic syndrome up till now, changing life style is the only alternative means for therapy and dietary intervention is considered as the most important strategy for this purpose [15]. Three areas are the targets: 1- Reduction of obesity, 2- Increasing physical activity and 3- Nutritional intervention. It is worth mentioning that nutritional intervention has improved many disease states like diabetes mellites, depression, in addition to improving the performance of athletes as previously mentioned in several studies [16, 17, 18]. Improving systemic inflammation is also another new approach for managing MetS [19]. Among the foods that have anti-inflammatory activity are legumes with their bioactive peptides.

Legumes are of special interest owing to its well-established nutritional, environmental and economic benefits [20] and considered as the viable option for replacing animal-based protein to satisfy the increased demand for animal-based protein in developing countries and to lower the risk of animal-based protein increased consumption [21]. Their higher consumption is associated with lower risk for heart diseases, stroke, hypertension and type 2 diabetes mellitus [22]. Fava bean is one of the oldest legume crops that are cultivated worldwide [23].

Vicia faba L., which belongs to the family Fabaceae, and currently is termed as fava bean, is consumed widely in many countries. Its edible portion is the seeds. It is native to North Africa, Southwest Asia, Middle east countries and Europe [24]. Rahate et al. [25] reported that Mediterranean countries, Egypt, Ethiopia, India, China, Northern Africa and Northern Europe are the major fava bean producers. Fava bean has been regarded as a healthy, sustainable alternative for partially replacing animal protein sources in human diets [26]. The cooked fava bean or “Ful Medames” is an Egyptian staple food and considered as a national dish that is consumed as a breakfast by most of the Egyptians. Its dietary importance is consequent of its high valued protein content varying from 22.4 to 36.0% [27] as well as its bioactive compounds that give it the advantage of maintaining human health and preventing some diseases in addition to its being a low-cost food [21]. It contains considerable amounts of vitamins, complex carbohydrates, minerals, in addition to various bioactive components such as tocopherols, phenolic compounds, triterpenic acids, hemagglutinin, trypsin inhibitors and L-dopa. These compounds are known to have various health benefits such as anti-inflammatory, antioxidant, anti-obesity, anticancer, lowering cholesterol levels, hepatoprotective effects and the treatment of Parkinson’s disease [27].

Since nutritional intervention is a key determinant in managing metabolic syndrome, thus introducing a nutritive meal with many benefits such as antioxidant and anti-inflammatory may be a step in the way of managing metabolic syndrome. Thus, the aim of the present study is to evaluate the effect of cooked fava bean meal or “Ful Medames”, the most popular meal for Egyptians, on restoring metabolic disorders and inflammation accompanying to the induced metabolic syndrome in Wistar rats. Also, to evaluate its epigenetic relevance which is represented by changing protein expression of some white adipose tissue adipocytokines namely; resistin, leptin and adiponectin. To the extent of our knowledge, there is no single study that evaluates the impact of the consumption of an Egyptian national dish, "Ful medames," which is widely consumed in this form, on metabolic syndrome-associated

alterations and the present study would be the first study to do this.

2. Materials and methods

Materials

Fava bean (*Vicia faba)* was obtained from the Agricultural Research Centre in its ripening season. All other components of the meal which were; onion, turmeric, garlic, lentil, cumin, Table salt, lemon fruits and olive oil were purchased from the local market. Ingredients of the experimental diet for the feeding experiment were purchased as follows; cellulose, casein, cholesterol powder, bile salts and chemicals used to formulate the vitamin and salt mixtures as well as Folin-Ciocalteu reagent were all purchased from Laboratory Rasayan, Fine Chemical Limited, Mumbai, India. Other constituents of the experimental diet as well as lard were obtained from the local market. All solvents and standards used either for extraction or for characterization of the differential polyphenolic compounds of the prepared fava bean meal by HPLC or for histopathological examination as well as DPPH and TPTZ reagent used for the estimation of the antioxidant activity of the prepared meal and the streptozotocin (STZ) all were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. (St. Louis, Mo, 63103, USA).

Kits used for the determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, triglycerides, total cholesterol, and high-density lipoprotein cholesterol (HDL-C) were purchased from Salucea Co., Netherlands, while the kit used for determination of serum creatinine was obtained from Erba Co., Italy. Kits used for determination of serum glucose and blood haemoglobin were purchased from Biodiagnostic CO., Egypt. Kits used for determination of serum insulin and c-reactive protein (CRP) by ELISA technique with catalogue numbers of: E-EL-R3034 and E-EL-R0506, respectively were obtained from Elabscience Co., China.

Chemicals and kits used for genetic analysis were as follows: RNA stabilizer reagent solution (Qiagen, Germany), primers for each of leptin, resistin and adiponectin as well as β-actin gene as a reference gene (Biosearch Technologies, USA), Maxima SYBR Green qPCR Master Mix, Gene JET RNA Purification Kit and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific Co., USA).

Animals used in this study were Wistar Albino rats of male sex. They were purchased from Central Animal House of the National Research Centre, Dokki, Egypt. The protocol for the animal experiment was approved by the Ethical Committee of the National Research Centre with an approval no. of "19/181".

Methods

Technological treatments

Preparation of fava bean before cooking

Fava bean seeds were soaked in warm water with a temperature of 37°C for 24 hours prior to cooking to get rid of the antinutritional factors and to shorten the time required for cooking. In addition, soaking helps to magnify the polyphenolic content of the seeds as previously reported by Siah *et al*. [28].

Preparation of the ready-to-eat meal from the cooked fava bean

After soaking, the water was decanted and the fava beans were allowed to be washed well. Then, a suitable volume of water was added to the fava bean seeds and the other additives were put which were; 10 g lentil, one fruit of lemon, 3 g garlic, 5 g onion and 1 g turmeric for each 100 g fava beans, all ingredients were added in a suitable cooking pan and covered and it was allowed to be cooked well. Then, spices were added to the cooked meal as Table salt, olive oil and cumin and mixed well. The cooked meal with additives (CFBM) was put in a sterilized jar and closed well under sterilized conditions. It was kept in the refrigerator until being used. This product is considered as a ready-to-eat meal and can be marketed. Then, the previous procedures were repeated but with no addition of any additives, just the table salt was added to obtain the cooked fava bean meal with no additives (CFBM-NA) to compare it with the cooked fava bean meal with additives (CFBM) to obtain a randomized control trial.

Air oven-drying

The two cooked meals (CFBM and CFBM-NA) were then allowed to dry at 50 °C in an air-ventilated oven until complete dryness. Finally, the dried meals were milled by a food miller (Mianta, Egypt). A small portion of the obtained powder was separated to be subjected to the extraction procedure for further determination of each of the antioxidant activity, the total polyphenolic content and the differential polyphenolic content by HPLC. The other portion of the milled meal (CFBM) was kept in the freezer at -20 °C until being used for the feeding experiment.

Determination of phenolic compounds and antioxidant activity

Extraction of the cooked meals

A weight of 1 g of each of the previously dried milled meals was added to 10 ml of 70% ethanol followed by sonication at 37°C for 1 hour. Then, the obtained suspension was centrifuged for 30 min at 3000 rpm followed by filtration. The filtrate volume

was completed to 25 ml by ethanol [29]. This solution was used to determine the total and differential polyphenolic compounds as well as the antioxidant activity.

Determination of the total polyphenolic compounds in cooked meals

Total polyphenolic content was assayed in the ethanolic extract for both cooked meals using the Folin-Ciocalteu assay as stated in the procedures reported by Ramful *et al*. [30].

Characterization and quantification of phenolic compounds in cooked meals by HPLC

The differential phenolic compound pattern in the ethanolic extract of each of the prepared meals was detected quantitively by HPLC technique as follows:

HPLC conditions

HPLC analysis was performed using an Agilent 1260 series. The separation was carried out using Eclipse C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A) ; 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C. Then, the obtained peaks of the phenolic compounds were compared with their corresponding peaks of the standards with known concentration and the concentration of each of the polyphenolic compounds was calculated.

Determination of antioxidant activity of the cooked meals

1. Determination of the antioxidant activity by ferric reducing power (TPTZ) assay

The ferric reducing power (TPTZ) assay is based on the ability of phenolics in each extract to reduce Fe3+ to Fe2+ and it was carried out according to de Moraes Barros *et al*. [31]. The analysis was performed in triplicate, using an aqueous Trolox solution as standard and the results were expressed as µg Trolox equivalents/g of dry weight sample.

2. Determination of the antioxidant activity by DPPH

The radical scavenging activity for both extracts was measured by the DPPH (1, 1-diphenyl-2-picrylhydrazyl) method as described in the procedures carried out by Hayat *et al*. [29]. It was detected for each sample by measuring the decrease in absorbance, then, according to the following equation, the scavenging capacity was calculated:

$$\text{Scavenging activity (\%)} = \frac{Ac - As}{Ac} \quad X\ 100$$

Where Ac and As refer to the control and the sample absorbance, respectively at a wavelength of 517 nm.

L-ascorbic acid solution was also measured by the DPPH method for its antioxidant activity and used as a standard. Then, the antioxidant values of samples were expressed as $mg.g^{-1}$ dry weight L-ascorbic acid equivalent antioxidant capacity.

Sensory evaluation

Sensory evaluation of the cooked fava bean meal with additives (CFBM) or Ful Medames was done and compared to the cooked fava bean meal with no additives (CFBM-NA) by a number of 20 well-trained panelists aged between 30 and 50 years that were selected to take part in the sensory panel in the Food Technology Department, Institute of Food Industries and Nutrition, National Research Centre, Egypt. The panelists measured the selected attributes for each cooked meal such as color, taste, odor, flavor and overall-acceptability according to Mahmoud *et al.* [32]. All evaluations were performed in a well-controlled laboratory. It was evaluated using a 10-point hedonic scale as following: 1= dislike extremely, 2 = mostly dislike, 3= dislike very much, 4 = dislike moderately, 5 = neither like nor dislike, 6 = like, 7 = like moderately, 8 = like very much, 9 = mostly like, 10 = like extremely. The rating scale was used for all sensory evaluation parameters according to the method described by Larmond [33].

Biological Evaluation

Cooked fava bean meal (CFBM) was biologically evaluated in an experimental model of induced metabolic syndrome. Twenty-four male Wistar albino rats were used with body weight ranging from 170 to 190 g. The procedures of the animal experiment were done in compliance with the guidelines stated by the Ethical Committee of the National Research Centre. Eight rats out of twenty-four were separated and considered as the control negative group and fed on the semisynthetic diet which was prepared according to Revees *et al*. [34]. Then, the rest of the animals were induced for metabolic syndrome. This model of metabolic syndrome was done as modified from Suman *et al.* [35], Gancheva *et al.* [36] and Mahmoud *et al.* [32].

Rats were fed on a high fat and cholesterol diet (20% lard, 2% cholesterol powder and 0.25% bile salts) for eight weeks to induce dyslipidemia. ON the seventh week, rats were intraperitonially injected with streptozotocin (STZ) dissolved in citrate buffer (0.1 mol/L, pH 4.5) which was freshly prepared just before injection at a dose of 40 mg/kg B. wt. to induce hyperglycemia. Then, the feeding experiment started as follows:

Group 1: A control negative group fed on the basal semisynthetic diet.

Group 2: A control positive group induced for metabolic syndrome by feeding on the basal semisynthetic diet containing 200 g lard + 20 g cholesterol +2.5 g bile salts for each kg diet (HFHC) and was intraperitoneally injected with 40 mg/kg B. wt. of STZ by the end of the seventh week.

Group 3: A group previously induced for metabolic syndrome for 8 weeks by feeding on HFHC diet and intraperitoneally injected with 40 mg/kg B. wt. of STZ by the end of the seventh week, then beginning from the ninth week, this group was supplemented with cooked fava bean meal (CFBM) as 200 g for each Kg of the HFHC diet.

Then, the feeding experiment lasted for further four weeks. At the end of the experimental period, all animals were sacrificed by intraperitoneal injection of pentobarbital (300 mg kg^{-1} B. W.). Fasting blood samples were withdrawn from the suborbital vein into three tubes for each sample containing either heparin or sodium florid for the determination of blood hemoglobin and blood glucose, respectively and the third was empty for serum for determination of the other biochemical parameters. The serum was separated by centrifugation at 4000 rpm for 15 min and stored in many aliquots for each sample at – 80 °C for further biochemical analysis.

Liver, heart and pancreas from each rat were separated, washed with saline and dried and the liver was weighed for each rat and recorded to calculate the hepatosomatic index (HI) = liver weight / final body weight. Then, all separated organs were immersed in 10% formalin solution for further histopathological examination. Visceral adipose tissue was collected under liquid nitrogen from each rat for gene expression analysis.

Biochemical analysis

Hemoglobin concentration was assessed as described by Betke and Savelsberg [37]. Blood glucose was estimated according to Trinder [38]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed for their activities by the method of Henry *et al*. [39]. Serum urea and creatinine were detected as described in the methods of Fawcett and Scott [40] and Murray [41], respectively. Lipid profile was detected as follows; serum triglycerides (TG) was estimated according to Scheletter and Nussel [42], serum total cholesterol was assessed as described by Meiattini *et al*. [43], serum HDL-C was determined according to the method of Grove [44]. LDL-C was calculated as described by Warnick *et al* [45] by the following equation:

LDL-C = Total cholesterol – (HDL-C+ VLDL-C)

Where, VLDL-C = Triglycerides /5

The optical density of all aforementioned biochemical parameters was measured by a colorimetric technique using a spectrophotometer (Shimadzu UV-2401 PC, Australia). Then, their concentration was calculated.

The concentration of insulin and C reactive protein (CRP) were determined according to the manufacturer's instructions for each of them by the ELISA technique using a device of ELISA reader with a model of Sunrise, Tecan Austria GmbH 5082 Grödig, (Austria). Then, the insulin resistance (HOMA-IR) was calculated according to Bose et al. (2008) as follows;

HOMA- IR = fasting blood glucose (mmol.L-1) X insulin ($mU.L^{-1}$) / 22.5

Gene expression analysis

Assessment of gene expression of each of leptin, adiponectin and resistin was done by qRT-PCR technique (quantitative Real Time Polymerase Chain Reaction) that was carried out as follows:

RNA extraction and cDNA synthesis

One hundred mg of visceral adipose tissue was obtained from each rat and stored up to 4 days maximum in the RNA stabilizer solution (RNA later reagent) obtained from Qiagen, Germany. Then, about 30 mg of each sample were homogenized in a mortar under liquid nitrogen. Isolation of RNA from tissue was carried out as described previously in the method of El-Moghazy et al. [46] and by the use of a Gene JET RNA purification kit obtained from Thermo Scientific Company, (USA). Quantification of RNA was done using an ND-1000 Spectrophotometer (NanoDrop, USA). Then, 1 µg sample of tissue-derived RNA was used to obtain cDNA (high-capacity cDNA) with a RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA). The incubation was done on the gradient

thermal cycler (Bio-Rad T100, Michan, USA) as described in the manufacturer instructions.

Quantitative Real Time Polymerase Chain Reaction

The quantitative real time polymerase chain reaction (qRT-PCR) was performed using the Stratagene Mx3000P Real-Time PCR System, Agilent Technologies (USA). It was carried out for each sample in duplicate using Maxima SYBR Green qPCR Master Mix, Thermo Scientific. The total volume of reaction mixture was 20 µL including 10 µL of SYBR Green Master Mix, 300 nmol/L of each primer set for each gene and 4 µL of cDNA (100 ng/µL) and completed to 20 µL with nuclease-free water. The gene expression for each gene was normalized to the β-actin gene as a reference gene.

The primer sequence for adiponectin gene was: forward primer 5′-AATCCTGCCCAGTCATGAAG-3′ and reverse one 5′-CATCTCCTGGGTCACCCTTA-3′ and for leptin gene was: forward primer 5′-CTCAGCATTCAGGGCTAAGG-3′ and reverse primer 5′-AAGCCTCGCTCTACTCCACA-3′, while for resistin gene was: forward primer 5′-AGTTGTGCC-CTGCTGAGCTCTCTGCC-3′ and reverse primer 5′-CCCATTGTGTATTTCCAGACCCTC-3′ and for the reference gene "β-actin" was: forward primer 5′-GTGGGGCGCCCCAGGCACCA-3′ and reverse primer 5′-CTCCTTAATGTCACGCACGATTTC-3′. These gene primers were requested and synthesized by Biosearch Technologies, USA). The thermal cycler program was: 95 °C for 5 min then, 40 cycles at 94 °C for 15 s, then annealing of each gene for 60 s at its suitable melting temperature as follows: 62°C (for leptin), 65°C (for adiponectin), 62°C (for resistin) and 60°C (for β-actin) and finally, extension at 72 °C for 10 s. Then, the method of relative quantification of mRNA; the $2^{-\Delta\Delta CT}$ formula was applied to determine the fold difference in gene expression.

Histopathological examination

Liver, heart and pancreas specimens from all experimental rats were collected, fixed in neutral buffered formalin 10%, washed, dehydrated, cleared and embedded in paraffin. The paraffin embedded blocks were sectioned at a 5-micron thickness and stained with Haematoxylin and Eosin for light microscopic examination (Olympus BX50, Tokyo, Japan) [47]. Histopathological damages were graded from (0- 3) as (0) indicated no change, (1), (2) and (3) indicated mild, moderate and severe changes, respectively, while the grading was determined by percentage as follows: (<30%) showed mild changes, (<30% – 50%) indicated moderate changes and changes more than 50% (>50%) indicated severe changes [48].

Statistical analysis

All data were analyzed using the computerize program package "SPSS" for windows, version "26" (SPSS Inc., Chicago, IL, USA) and GraphPad Prism statistical program, version "6.0" (GraphPad Software, Inc., San Diego, CA, USA). The one-way analysis of variance; "ANOVA" test was carried out for comparing means of multiple groups followed by Dunkn post hoc test. Also, independent T-test was used to compare data in some parameters including the antioxidants in the prepared meals and the sensory evaluation of these meals. All values were represented as mean ± S.E. Values with a "p" value less than 0.05 were considered significant, otherwise it was not significant.

3. Results and discussion

Many of the staple dishes that are prepared from pulses can be cooked in different ways like fava beans or may be called broad beans which is served as Ful Medames in Egypt [28]. This meal is the most popular and common meal for the Egyptians. Most of Egyptians have this meal for their breakfast. Epidemiological studies revealed that the intake of legumes and whole grain cereals is correlated with reduced risk for many chronic diseases like diabetes mellitus, cardiovascular diseases and cancer [49].

The chemical composition of fava bean meal with additives as shown in Table (1) showed that the protein content of the CFBM which accounts for 26.56% is relatively high, thus this meal can be considered as a good source of dietary protein. Moreover, Labba *et al.* [50] reported that the protein content of *Vicia faba* for different cultivars ranges from 22.7 to 28.1 g protein for each 100 g dry weight of fava beans which is in accordance with the obtained data of the present study.

In addition, the proportions of the other constituents of the CFBM are good, reflecting that this meal is considered as a balanced meal providing that the source of fat content is olive oil, which is the recommended fat source in the Mediterranean diet that is considered as the healthiest dietary pattern for avoiding many diseases like cardiovascular diseases, metabolic syndrome, hypertension and diabetes [51].

Table 1: The proximate composition of the cooked fava bean meal as percentage.

	Protein%	Fat%	Ash%	Moisture%	Carbohydrate%
CFBM*	26.56	8.07	3.36	8.58	53.43

*CFBM: cooked fava bean meal with additives.

Polyphenols are substances that are synthesized in plants to carry out various functions such as protection from microbial infections as well as having some therapeutic benefits for humans. They are strong antioxidants [52] and have anti-inflammatory effects [53], thus can be used owing to their therapeutic importance for many diseases in humans. In this study, the total polyphenols of the prepared meals as detected by the Folin-Ciocalteu assay illustrated the presence of a considerable amount of polyphenols (Table 2) with a significant increase for the cooked fava bean meal with additives with a value of 19.60 ± 0.30 compared to a value of 8.72 ± 0.06 for the cooked fava bean meal with no additives. These polyphenols of the CFBM with additives are not of the fava bean itself but also due to those found in the other constituents of the prepared meal like lemon juice, spices and olive oil. Thus, it can be said that these natural additives magnified the bioactivity of the prepared meal. Also, it is worth mentioning that fava bean was soaked before cooking to get rid of the antinutritional factors, to shorten the cooking time and to magnify its bioactive components as it increases its polyphenolic content [28, 54]. A considerable antioxidant activity of the CFBM was recorded as they were detected by the two tests; DPPH and TPTZ (Table 2). This antioxidant activity can be attributed mainly to the polyphenols. Siah et al. [28] mentioned that fava bean as well as many other pulses have a high content of polyphenols and possess antioxidant activity. From the data showed in Table (2), it is noticed that the antioxidant activity either that represented by DPPH or that represented by TPTZ for the CFBM with additives is significantly higher than those for the CFBM with no additives by 1.5 to 2 folds illustrating the role of these natural additives in increasing the antioxidant activity of the prepared meal.

Seventeen polyphenols were detected by HPLC (Table 3) with relatively higher amounts for the CFBM with additives compared to that of CFBM with no additives. Polyphenols contribute to the overall antioxidant activity of plant foods [55]. Fava bean is considered as a good source for polyphenols. Many health benefits were reported for these polyphenols that were detected in this study, among which; potent antioxidant, anti-inflammatory, lipid and carbohydrate metabolism regulation, anticarcinogenic, antidiabetic, cardioprotective, improve insulin resistance and atherosclerosis, nephroprotective and antimicrobial as well as the impact on obesity by reducing body weight in obese animals due to lowering the accumulation of hepatic triacylglycerols. All these benefits were mentioned for chlorogenic acid, ellagic acid, gallic acid, caffeic acid and rutin [53, 56 - 60]. Also, Noce et al. [61] mentioned that catechin compounds are among the most potential natural bioactive compounds with anti-obesity action and the prepared meals in the present study are catechin rich as seen in Table (3), thus can be beneficial in combating the metabolic syndrome as the obesity is one of the prominent criteria for the metabolic syndrome. Moreover, quercetin is among the polyphenolic compounds in the prepared meals of the present study with a concentration in the CFBM with additives three folds higher than that in the CFBM with no additives reflecting the vital role of these additives. Quercetin was reported previously to have many benefits among which is the body weight reduction and also it acts as a tissue specific anti-inflammatory improving the systemic low-grade inflammation [61], thus can participate in mitigating the inflammation accompanying the metabolic syndrome.

In the present study, to have a very good taste and to increase the palatability of the meal as well as to increase its nutritional value, many additives were added like turmeric, onion, garlic, tomatoes during cooking and then, spices were added like cumin. Moreover, lemon juice was added and olive oil.

The previously mentioned additives gave the meal a very good palatability as shown in Table (4) for sensory evaluation of the meal, in which the overall acceptability of the meal is very high accounting for 9.29 ± 0.20 out of ten for the CFBM with additives which is significantly higher compared to the CFBM with no additives which was 7.20 ± 0.24 reflecting the role of these natural additives in improving the overall acceptability of the CFBM.

As shown in Table (5), hyperglycemia was noticed in the control positive group with a a serum glucose level of 200.24 ± 7.87 mg/dl when compared to the control negative group; 92.36 ± 6.72 mg/dl which was reduced significantly to some extent in the group that was fed on the cooked fava bean meal with a serum glucose concentration of 161.15 ± 2.60 mg/dl.

Table 2: Total polyphenols (as gallic acid) & antioxidant activity by DPPH (mg ascorbic acid eq/g d. w.) and TPTZ (µg Trolox eq/ g d. w.) of the cooked fava bean meals.

Sample	Total polyphenols (mg /g d. w.	DPPH (mg ascorbic acid eq/g d. w.	TPTZ (µg Trolox eq/g d. w.)
CFBM-NA	8.72 ± 0.06	195.06 ± 2.53	310.04 ± 7.86
CFBM	19.60 ± 0.30*	376.32 ± 11.40*	477.70 ± 6.13*

CFBM: NA: cooked fava bean meal with no additives, while CFBM: cooked fava bean meal with additives. Data are represented as mean ± SE. Values that have (*) are significant with their corresponding values at the same column.

Table 3: Concentration of polyphenols in the cooked fava bean meals by HPLC.

	Concentration	
Polyphenolic compounds	**CFBM-NA (µg/g d. w.)**	**CFBM (µg/g d. w.)**
Gallic acid	19.29	12.91
Chlorogenic acid	25.44	53.44
Catechin	21.20	25.71
Methyl gallate	2.95	5.86
Caffeic acid	69.73	89.08
Syringic acid	2.96	5.69
Rutin	4.09	28.35
Ellagic acid	40.74	63.63
Coumaric acid	0	3.12
Vanillin	1.82	1.64
Ferulic acid	1.87	5.48
Naringenin	2.51	2.56
Daidzein	0.79	0.66
Qurecetin	3.00	10.40
Cinnamic acid	0.58	1.06
Apigenin	0	5.86
Hesperetin	5.33	0

CFBM: NA: cooked fava bean meal with no additives, while CFBM: cooked fava bean meal with additives

In fact, this result for the control positive group was expected, since it was fed on a high fat diet and injected with a low dose of streptozotocin, which is considered a method to induce type 2 diabetes mellitus in some studies [35, 62], beside its being a method for induction of metabolic syndrome in other studies [36]. Anyhow, CFBM was able to counteract to some extent this hyperglycemia. In addition, a significant reduction in serum insulin concentration was noticed in the metabolic syndrome-induced group with a value of 197.39 ± 22.6 pg/ml compared to a control negative value of 336.8 ± 5.13 pg/ml, but this reduction was restored in the metabolic syndrome-induced group that received the CFBM to become 332.79 ± 5.09 pg/ml (Fig 1-A). On the other hand, a non-significant increase was recorded for insulin resistance or HOMA-IR for the control positive group which was2.46 ± 0.38 compared to a control negative of 1.92 ± 0.17, however this increase was slightly decreased in the group with metabolic syndrome and fed on the CFBM with a value of 2.32 ± 0.18 (Fig 1-B). In fact, hyperglycemia and increased insulin resistance are two of the metabolic syndrome criteria [2]. Also, Li *et al*. [63] reported that increased insulin resistance is associated with several diseases among which the metabolic syndrome. The significant reduction of blood glucose by 18.78% in the metabolic syndrome group that received the CFBM can be explained on the basis of the presence of polyphenolic compounds in fava beans as detected by HPLC in the present study namely; chlorogenic acid, ellagic acid and rutin which were said to have antidiabetic effects as well as improving insulin resistance [56, 60]. Furthermore, the antioxidant activity of the polyphenols that were detected in fava beans per si is able to counteract the increased oxidative stress leading finally to reduce the increased blood glucose level as mentioned previously by El-Shobaki et al. [62] and Mahmoud et al. [64].

A significant drop in blood hemoglobin concentration was noticed in the control positive group compared to the control negative group (Table 5), but this drop was improved to some extent in the group with metabolic syndrome that was fed on the CFBM indicating that a positive impact was achieved by feeding the metabolic syndrome rats on CFBM. A significant drop in blood hemoglobin concentration was noticed in the control positive group compared to the control negative group (Table 5), but this drop was improved to some extent in the group with metabolic syndrome that was fed on the CFBM indicating that a positive impact was achieved by feeding the metabolic syndrome rats on CFBM. It is worth mentioning that fava bean is a rich plant source for protein as mentioned in Table (1), minerals (sodium, potassium, calcium, copper, zinc, iron, manganese, magnesium, phosphorus, and sulfur) and vitamins like folate [65]. All these aforementioned food staffs and trace elements are of great importance in ameliorating decreased blood hemoglobin concentration.

On the other hand, an increased activity was recorded for liver transaminases; ALT and AST of the control positive group (Table 5), this increase was insignificant in case of AST and significant in case of ALT, but the group with metabolic syndrome that was fed on the CFBM showed improvement in liver transaminases.

Table 4: Sensory evaluation of the cooked fava bean meals without and with additives.

	Parameters				
Sample	**Color**	**Taste**	**Odor**	**Flavor**	**Overall acceptability**
CFBM-NA	7.15 ± 0.22	7.70 ± 0.23	7.00 ± 0.18	7.35 ± 0.17	7.20 ± 0.24
CFBM	9.5 ± 0.13*	9.42 ± 0.20*	9.14 ± 0.23*	9.57 ± 0.20*	9.29 ± 0.20*

CFBM: NA: cooked fava bean meal with no additives, while CFBM: cooked fava bean meal with additives. Data are represented as mean ± SE. Values that have (*) are significant with their corresponding values at the same column.

Table 5: Blood hemoglobin, serum glucose, ALT, AST, urea, creatinine, total cholesterol, HDL-C, LDL-C, triglycerides, body weight change and hepatosomatic index of all groups.

	Groups		
Parameters	**Control negative**	**Control positive**	**CFBM***
Hb (g/dl)	12.29 ± 0.47 [b]	10.26 ± 0.09 [a]	11.51± 0.57 [a b]
Glucose (mg/dl)	92.36 ±6.72 [a]	200.24 ±7.87 [c]	161.15 ± 2.60 [b]
ALT (U/L)	25.83 ± 3.19 [a]	39.97 ± 1.79 [c]	33.00 ± 1.51[b]
AST (U/L)	45.25 ± 5.22 [a]	54.00 ± 3.40 [a]	49.60 ± 3.87 [a]
Urea (mg/dl)	37.44 ± 2.73 [a]	60.87 ± 2.69 [b]	35.42 ± 0.33 [a]
Creatinine (mg/dl)	0.79 ± 0.06 [a]	0.52 ± 0.01 [a]	0.60± 0.06 [a]
TC (mg/dl)	92.41 ± 5.67 [a]	254.80 ± 11.06 [c]	199.35 ± 13.28 [b]
HDL-C (mg/dl)	45.67 ± 3.78 [b]	33.87 ± 2.71 [a]	41.33 ± 1.28 [ab]
LDL-C (mg/dl)	29.97 ± 2.84 [a]	194.27 ± 11.40 [c]	139.72 ± 13.26 [b]
TG (mg/dl)	82.50 ± 7.49 [a]	115.17 ± 10.02 [ab]	92.33 ± 6.65 [ab]
B wt gain (g)	55.86 ± 2.63 [a]	53.00 ± 3.24 [a]	61.67 ± 2.68 [a]
H I	3.23 ± 0.12 [a]	7.07 ± 0.05 [c]	6.42 ± 0.25 [b]

*CFBM: cooked fava bean meal. HI: hepatosomatic index. Values are represented as mean ± SE and $P < 0.05$ was considered as the level of significance. Values sharing the same letters at the same raw are non-significant, while values sharing different letters at the same raw are significant.

The increased activity of transaminases can be attributed to the effect of the high fat diet which leads to the accumulation of fat in hepatocytes affecting negatively their ability to perform their functions normally and also increases oxidative stress that in turn affects the integrity and permeability of the hepatocytes' cellular membrane due to increased lipid peroxidation of the membrane which finally causes leakage of the transaminases from hepatocytes into the blood stream leading to their elevation [66]. However, the CFBM with its content of polyphenols including gallic acid, ellagic acid, caffeic acid and rutin in addition to the other polyphenols with their antioxidant activity, as reported in many studies [53, 57, 58, 60], was able to counteract the increased oxidative stress, thus restoring transaminases activity in serum.

On the other hand, a significant rise in urea concentration was noticed for the control positive group compared to the control negative group (Table 5), but the group with metabolic syndrome that was fed on the CFBM showed restored urea concentration, while, there was no significant change in serum creatinine among the studied groups. As mentioned above, the high fat diet caused a state of increased oxidative stress, which in turn affects the nephrotic tissue negatively leading to increased serum urea concentration in the control positive group [67], however, the CFBM with its polyphenolic content that have antioxidant activity [53, 57, 58, 60] could counteract the increased oxidative stress leading to restoring serum urea concentration.

Table (5) shows also the lipid profile of the studied groups illustrating a state of dyslipidemia for the metabolic syndrome-induced group with a highly significant increase for each of the serum total cholesterol (TC), triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C), while a highly significant reduction in high-density lipoprotein cholesterol (HDL-C) was noticed. This altered lipid parameters (TC & LDL-C) were highly significantly improved, while TG & HDL-C were improved but non-significantly in the group with metabolic syndrome that was fed on the CFBM. The change that occurred in lipid parameters was simply and directly a consequent result of increased intestinal fat absorption due to increased fat intake as a result of feeding rats on a high fat and cholesterol diet [32]. However, the CFBM was able to counteract to some extent these altered lipid parameters owing to its antioxidant activity. In addition, Singh *et al.* [68] stated that *Vicia faba* is a very good source for dietary fiber which hinders intestinal fat absorption

as mentioned previously [69], which is another proposed mechanism for restoring to some extent the altered lipid parameters. In addition, some of the detected polyphenols of the prepared CFBM in the present study which were the catechin compounds were reported to decrease the intestinal absorption of the lipid [61]. No significant change was noticed in the body weight among different groups (Table 5), but a significant increase in the hepatosomatic index (HI) was recorded for the control positive group, which was restored significantly in the metabolic syndrome group that was fed on the CFBM. The increased liver weight % may be due to the accumulation of triacylglycerols in hepatocytes as a result of increased intestinal fat absorption as mentioned previously by Nooman et al. [70]. Feeding the metabolic syndrome-induced group on the CFBM with its high content of dietary fibers [28] was able to hinder intestinal fat absorption and hence to reduce fat accumulation in hepatocytes leading to a reduction in HI in this group.

An inflammation that was proved by the significant increase in c-reactive protein proinflammatory cytokine in the metabolic syndrome-induced group with a value of 61.34 ± 3.62 ng.ml-1 compared to a control negative value of 42.05 ± 0.68 ng.ml-1 which was more or less restored in the diseased group that received CFBM recording a value of 55.37 ± 2.09 ng.ml-1 as shown in Fig 1-C. A state of inflammation was recorded previously, which was concomitant to feeding rats with high fat and cholesterol diet and represented by the increased proinflammatory cytokine; TNF-α (tumor necrosis factor alpha) as mentioned by Mahmoud et al. [32]. As mentioned above that the high fat and cholesterol diet causes a state of increased oxidative stress. In this respect, Tajik et al. [56] mentioned that Inflammation which is closely correlated to increased oxidative stress and reactive oxygen species, is observed in considerable amounts in the signaling cascade that is involved in the process of inflammatory response. The antioxidant activity of the CFBM was able to counteract the increased inflammatory response reducing CRP in the group with metabolic syndrome that was fed on CFBM. Another explanation for this phenomenon is that most of the polyphenolic members of the fava beans including chlorogenic acid, ellagic acid, gallic acid, caffeic acid, naringenin and rutin have anti-inflammatory properties as mentioned in many studies [56, 58 – 60].

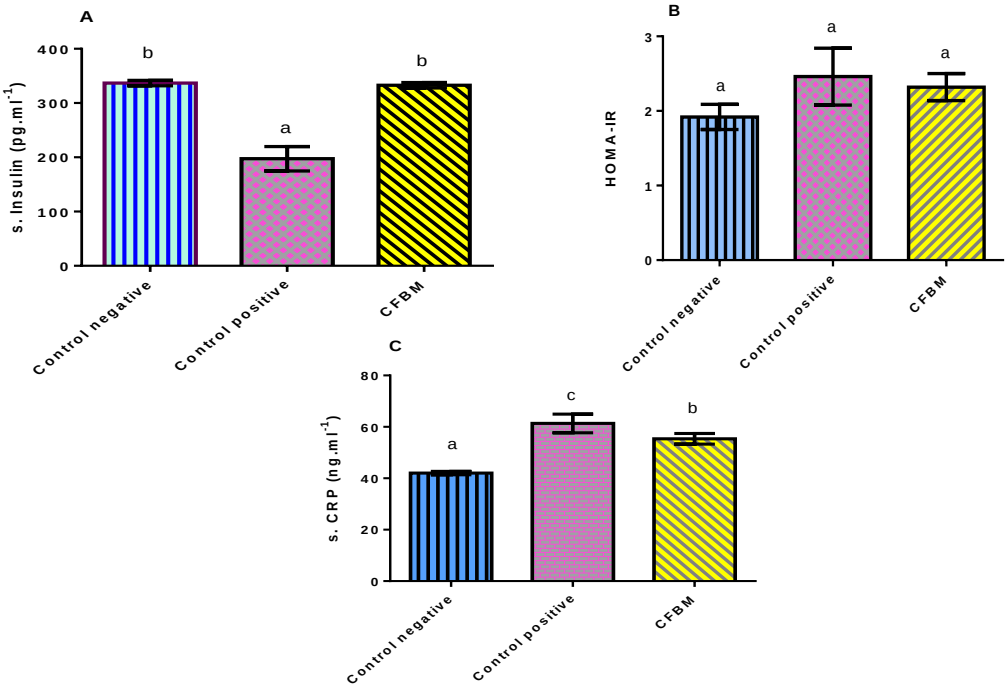

Fig. 1: A- Concentration of serum insulin of all studied groups. B- Insulin resistance (HOMA-IR) for all studied groups. C- Concentration of the inflammatory cytokine; CRP of all studied groups. CFBM: cooked fava bean meal. Columns that share the same letters reflect non-significant values, while columns that share different letters have significant values.

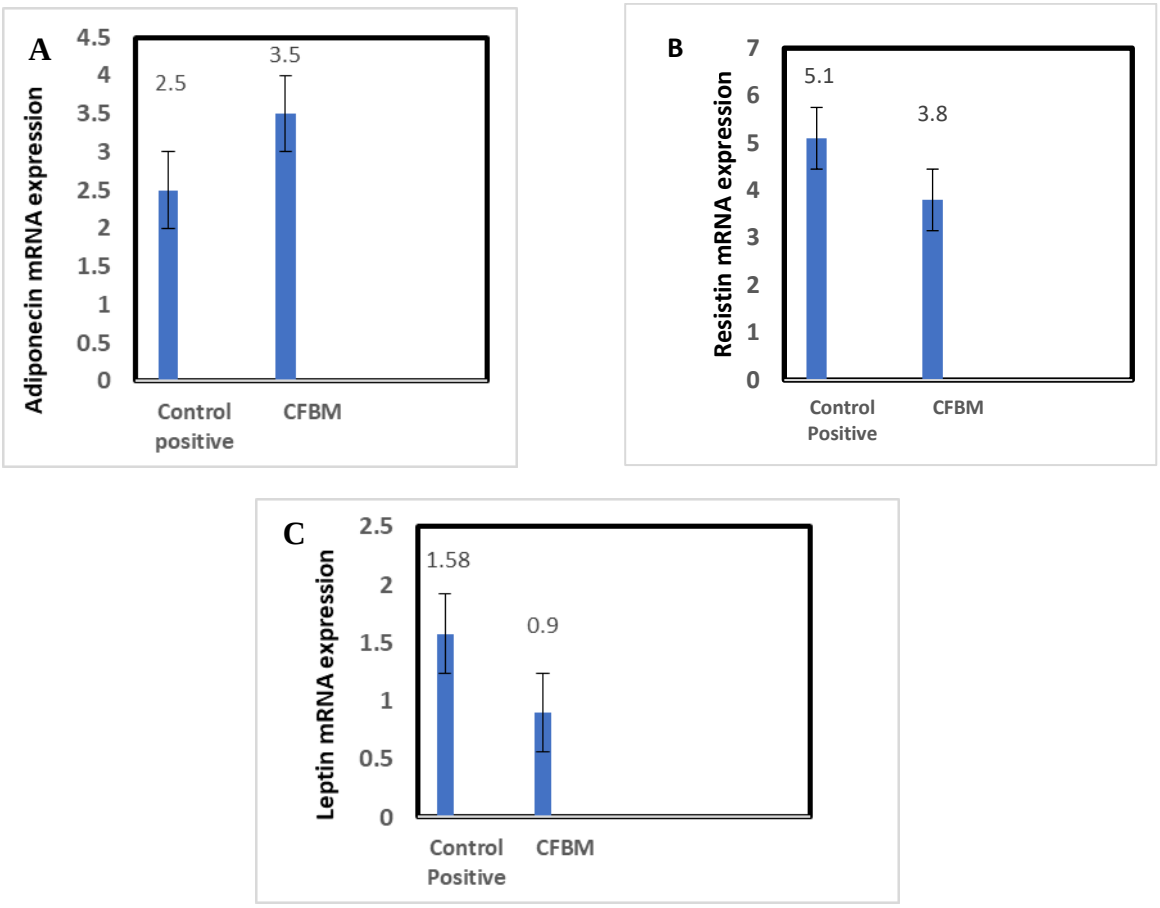

Fig. 2: Effect of feeding metabolic syndrome-induced rats with the CFBM on mRNA expresión of: A- Adiponectin gene. B- Resistin gene. C- Leptin gene. Data is represented as fold variation of the negative control.

Effect of feeding CFBM on adiponectin, leptin & resistin mRNA expression in adipose tissue of rats with metabolic syndrome

Comparative gene expression analysis by RT-PCR showed that adiponectin gene expression was upregulated in the supplemented group with CFBM reaching 3.49 ± 0.036 folds compared to the control positive group with a significant change ($P < 0.001$) as shown in Fig 2-A. While, resistin and leptin gene expression in CFBM supplemented group was down-regulated to 3.8 ± 0.15 folds and 0.89 ± 0.035 fold, respectively compared to the control positive group ($P < 0.001$) which recorded 5.1 ± 0.05 and 1.58 ± 0.02 for resistin and leptin as illustrated in Fig 2-B and Fig 2-C, respectively. Adiponectin, resitin and leptin are products of white adipose tissue or adipocytes. They play a very crucial role in lipid metabolism and are closely related to insulin sensitivity [5]. It was found that in human studies, the higher the resistin levels, the more the insulin resistance compared to normal individuals with normal resistin levels and normal insulin action [9]. This observation is in accordance with the obtained data of the present study in which resistin mRNA was upregulated in the control positive group indicating higher insulin resistance, then by feeding the CFBM, downregulation of this resistin mRNA was detected compared to the control positive group indicating that insulin resistance began to decrease which is a step in the way of metabolic syndrome amelioration. Also, Norata *et al.* [71] reported that increased plasma resistin concentration is correlated with metabolic syndrome. In accordance to our results, Padmalyam and Suto [72] mentioned previously that low plasma level of adiponectin is prevalent in metabolic syndrome patients. Aljafary and Al-Suhaimi [8] added that reduced levels of adiponectin are correlated with metabolic syndrome progress, insulin resistance and hypertension in children. Moreover, Similar results for leptin, resistin and adiponectin were reported by Waterman et. al. [73] who stated that fortification of the rat diet with moringa leaves has led to hyporesistenemic, hyperadiponectinemic, hypoleptinemic effects in female rats. They attributed these results to the potent antioxidant activity of moringa leaves that contains many polyphenols in addition to its content of vitamins, minerals, amino acids, carotenoids,

alkaloids. Moreover, in the present study, the obtained data for the effect of CFBM may be attributed to its antioxidant potency in addition to its content of vitamins, minerals, amino acids and bioactive peptides. Many theories were postulated to explain the relation between resistin and insulin resistance. They said that resistin has a role in the proinflammatory processes. Some *in vitro* studies found that resistin expression is the result of the proinflammatory cytokines production, like TNF-α (tumor necrosis factor-α) and IL-6 (6interleukin-6). Signaling pathway of TNF-α stimulates intracellular kinases which inhibit the signaling of insulin receptors through serine phosphorylation of IRS-1(insulin receptor substrate-1). Adiponectin and IL-6 also regulate insulin sensitivity by similar mechanisms [74]. Albarracín & Torres [7] reported that adiponectin is linked to abdominal obesity, insulin sensitivity, and changes in lipid parameters, specially reduced HDL-C levels. Moreover, leptin is said to be associated with inflammation as it induces the production of the proinflammatory cytokines [75]. In addition, it was said to be involved in inflammation associated with metabolic syndrome and it acts as a signal in regulation of insulin sensitivity [7].

Histopathology

Liver

Microscopic examination of the liver (Fig. 3) of normal control rats revealed the normal hepatic parenchyma architecture which consists of central veins and hepatocytes arranged in hepatic cords (Fig. 3-A). In contrast, the liver of control positive group rats exhibited remarkable histopathological alterations manifested by extensive hepatocellular vacuolar degeneration (Fig. 3-B), hepatocellular steatosis (Fig. 3-C) focal hepatic necrosis associated with inflammatory cell infiltration (Fig. 3) and portal infiltration with inflammatory cells (Fig. 3-D). However, the liver of rats from the metabolic syndrome group that was supplemented with CFBM showed improvement as represented by reduction in both hepatocellular vacuolar degeneration and hepatocellular microvesicular steatosis (Fig3-E & F).

Heart

Concerning heart, microscopic examination of heart (Fig. 4) of normal control rats showed the normal histological structure of cardiac myocytes (Fig. 4-A). On the contrary, heart of rats from the control positive group revealed severe histopathological changes described as vacuolization of the sarcoplasm of cardiac myocytes, focal necrosis of myocytes associated with inflammatory cell infiltration (Fig. 4-B), intermyocardial oedema (Fig. 4-C & D), inflam-matory cells infiltration (Fig. 4- C) and haemorrhage (Fig. 2-D). Meanwhile, improved sections were seen in rats of the metabolic syndrome group that was supplemented with CFBM, mild changes were reported as vacuolization of the sarcoplasm of some cardiac myocytes (Fig. 4-E) and congestion of intermyocardial blood vessels (Fig. 4-F).

Pancreas

Pancreatic sections from normal control rats (Fig. 5), exhibited the normal histological structure of islets of Langerhans and exocrine secretory acini (Fig. 5-A). On the other hand, the pancreas of rats from the control positive group showed marked vacuolation of cells of the islets of Langerhans and epithelial lining pancreatic acini (Figs. 5-B & C). However, examination of sections from rats of the metabolic syndrome group that was supplemented with CFBM revealed vacuolation of some cells of islets of Langerhans (Fig. 5-D), congestion of pancreatic blood vessels and slight vacuolation of epithelial lining pancreatic acini (Fig. 5-E).

Table (6) shows the histopathological lesion score in the studied groups, reflecting the positive impact and improvement that was achieved by feeding the CFBM to the group of rats with metabolic syndrome. As obvious, the heart tissue was completely restored, while a very slight histopathological changes were still found in the CFBM supplemented group for each of the liver and pancreas tissues, indicating the ability of this meal to ameliorate metabolic syndrome-associated metabolic disorders. In fact, the results obtained from histopathological examination reinforce the biochemical and gene expression results of the present study.

4. Conclusion

The obtained data indicate that a typical model for metabolic syndrome was achieved fulfilling more than one of the metabolic syndrome criteria which are: hyperglycemia, insulin resistance, high triglyceride concentration and reduced HDL-C concentration. Moreover, data indicate that there is an inflammation concomitant with the metabolic syndrome which was represented by increased c-reactive protein. These alterations were attributed to the upregulation of expression of adiponectin, leptin and resistin, since this dysregulation in expression was concomitant to the components of the metabolic syndrome and was correlated to the inflammatory response accompanying the metabolic syndrome.

Moreover, the recorded histopathological changes in this study confirmed all biochemical parameters. Dietary intervention used in this experiment, which was "Ful Medames" succeeded to restore to a good extent the altered metabolic parameters and to alleviate the inflammation accompanying the metabolic syndrome through adjusting the expression of the white adipose tissue mRNA of the hormones responsible for insulin sensitivity.

5. Conflict of interest

The authors declare that there is no conflict of interest.

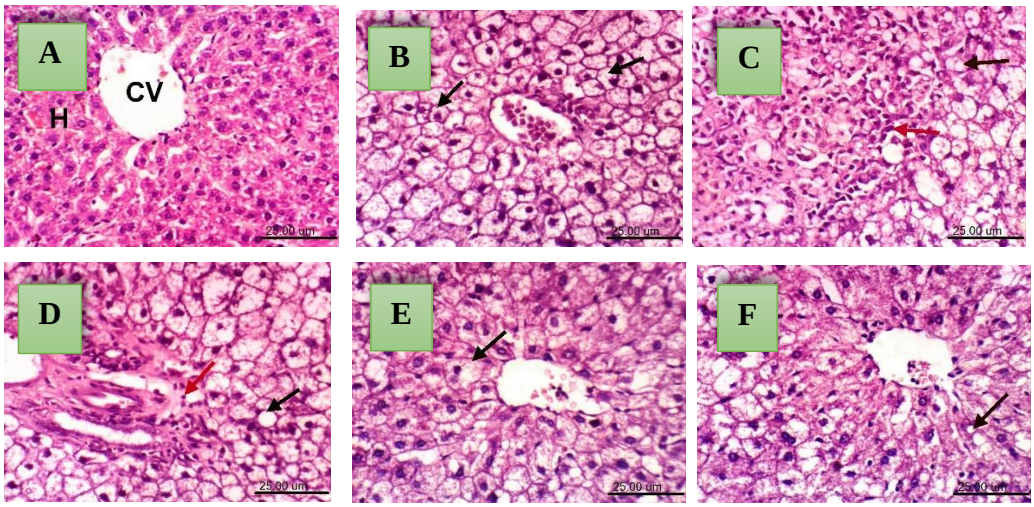

Fig. 3: Photomicrograph of rat liver from: (A) normal control group showing the normal histological architecture of hepatic parenchyma with central vein (CV) and concentrically arranged hepatocytes (H). (B) control positive group showing hepatocellular vacuolar degeneration (black arrow). (C) control positive group showing hepatocellular steatosis (black arrow) and focal hepatic necrosis associated with inflammatory cell infiltration (red arrow). (D) control positive group showing hepatocellular steatosis (black arrow) and portal infiltration with inflammatory cells (red arrow). (E) germinated fava bean supplemented group showing hepatocellular vacuolar degeneration (black arrow). (F) germinated fava bean supplemented group showing hepatocellular microvesicular steatosis (black arrow). (H & E, scale bar, 25um).

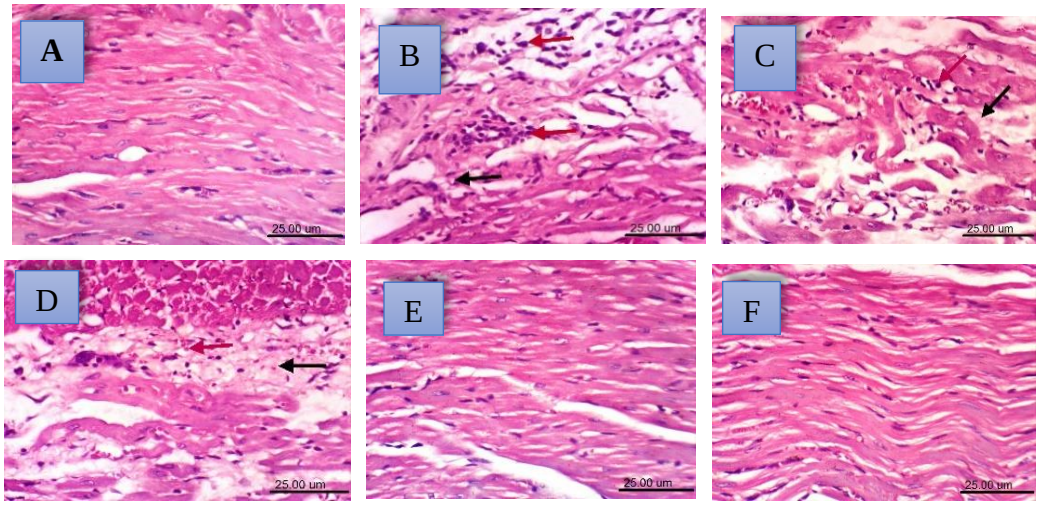

Fig. 4: Photomicrograph of heart: (A) Section of heart from a normal control rat showing the normal histological structure of cardiac myocytes. (B, C, D) Heart section of rat from control positive group showing: (B) vacuolization of the sarcoplasm of cardiac myocytes (black arrow) and focal necrosis of myocytes associated with inflammatory cell infiltration (red arrows), (C) intermyocardial oedema (black arrow) and inflammatory cells infiltration (red arrows), (D) intermyocardial oedema (black arrow) and haemorrhage (red arrow) (H & E, scale bar 25um). (E, F) Heart section of rat from germinated fava bean supplemented group showing showing no histopathological alterations (H & E, scale bar 25um).

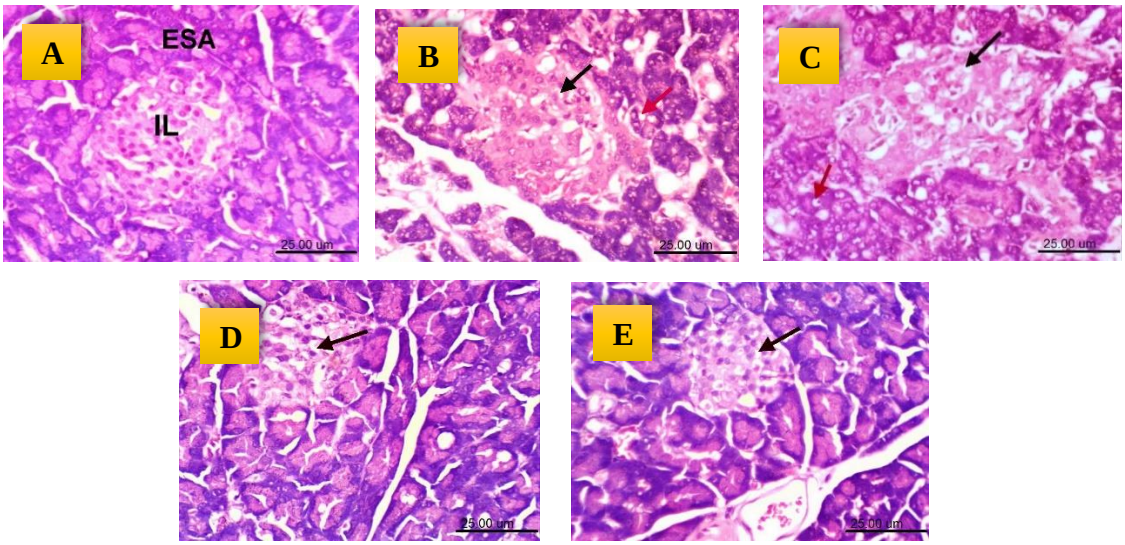

Fig. 5: Photomicrograph of the pancreas: (A) Section of pancreas from a normal control rat showing the normal histological structure of islets of Langerhans (IL) and exocrine secretory acini (ESA). (B, C) Pancreatic section of rat from control positive group showing vacuolation of cells of islets of Langerhans (black arrow) and epithelial lining pancreatic acini (red arrow) (D, E) Section of pancreas of rat from germinated fava beans supplemented group showing vacuolation of some cells of islets of Langerhans (black arrows). (H & E, scale bar 25um).

Table 6: Histopathological lesion scores in different experimental groups.

Organ	Lesions	Normal	Cont. +ve	CFBM
Liver	Hepatocellular vacuolar degeneration	0	3	1
	Hepatocellular steatosis	0	3	1
	Hepatocellular necrosis	0	2	0
	Inflammatory cell infiltration	0	2	0
Heart	Cytoplasmic vacuolization of sarcoplasm of cardiac myocytes	0	3	0
	Focal necrosis of cardiac myocytes & inflammatory cells infiltration.	0	2	0
	Intermyocardial oedema	0	3	0
	Congestion	0	2	0
	Haemorrhage	0	1	0
Pancreas	vacuolization of the cells of islets of Langerhan's	0	3	1
	Vacuolization of pancreatic acinar epithelium.	0	3	0

Histopathological damages were graded from (0- 3) as (0) indicated no change, (1), (2) and (3) indicated mild, moderate and severe changes, respectively.

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