# EFFECT OF MILLET FLOUR SUPPLEMENTATION ON SOME PHYSIOLOGICAL FUNCTIONS OF RATS FED ON HIGH-FAT DIET

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**Abstract**

Millet grains are nutri-cereals which have a high nutrient content. Excessive intake of a high-fat diet (HFD) leads to abnormal lipid metabolism, which further gives rise to various chronic diseases such as obesity, type 2 diabetes, heart disease and maybe cancer. The present study was conducted to investigate the effects of feeding supplementing HFD with millet flour (MF) on some physiological functions in rats. Thirty-five male rats were divided into five equal groups; the normal group (G1) was fed on a normal diet and the positive group (G2) was fed on HFD, while the other three groups were treated by feeding on supplemented diet with MF at levels of 5, 10 and 15%, respectively. Supplemented HFD with the different levels of MF significantly increased activities of antioxidant enzymes and serum HDL-c levels, and decreased body weight gain, feed efficiency ratio, serum levels of total lipids, triglycerides, total cholesterol, LDL-c, blood glucose, insulin, leptin, urea, creatinine, uric acid and MDA as well as the activities of liver enzymes (ALP, AST and ALT) and atherogenic index in obese rats, compared to that of the untreated obese rats. Histopathological examinations of liver and aorta also proved that supplemented diet with MF decreased the heart and aorta tissue damage in all treated rats. Conclusion: the present study concluded that millet grains may be healthful for patients with obesity. In addition, it improves many physiological functions, lipid levels and activities of antioxidant enzymes.

**Keywords**: Millet, Obesity, High-Fat Diet, Over Weight, Liver Functions, Antioxidant Enzymes.

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## 1. Introduction:

High-fat diets (HFD) are known to lead to a positive fat balance and consequently to adipose mass accumulation (**Flatt, 1995**). Also, these diets do not seem to stimulate fat oxidation rates in the same way in obese and lean subjects (**Westerterp et al., 2008**). The consumption of diets high in energy has become a habit in modern societies despite the reduction in daily energy needs (**Oggioni et al., 2014**). The preferences for high-fat diets lead to an obesogenic environment for humans, increasing the risk of developing diseases associated with metabolic disorders such as obesity and metabolic syndrome (MS). HFD induces obesity, hyperglycemia, hyperinsulinemia, hypertriglyceridemia, steatosis, beta-cell dysfunction and hypertrophy, and insulin resistance in muscle and liver (**Fontaine and Davis, 2015**). Aside from body image concerns, there are many chronic illnesses that are related to obesity, such as hypertension, joint pain, and acute myocardial infarction. They are all largely products of unhealthy chosen lifestyles and poor dietary habits (**Abady, 2020**). Therefore, an unhealthy diet is the major modifiable factor that can be targeted to control obesity, metabolic disease and other related diseases (**Elia and Cummings, 2007**).

Millets are a rapidly growing cereal grain that tends to be grown in many rural regions of Africa and countries such as China, India, etc. Most types of carbohydrates such as rice, bread, etc. tend to give a burst of energy in the beginning and then you need to eat again later to refuel. These carbohydrates tend to use up all their energy as soon as the body processes them, which is unhealthy for the body and causes imbalances such as storing the remaining unused glucose in fat, or causing high sugar levels (**Rao et al., 2017**). While millets help with homeostasis or balance within the body by adjusting the energy or glucose levels. Millets give an efficient system because they do not give the body as much glucose and their properties allow the body to use the glucose that was absorbed to be used in a very stabilized manner (**Rambabu, 2018**). In addition, millet as a kind of whole grain, has received much attention for its effects on metabolic diseases, including the prevention and mitigation of diabetes and obesity (**Li et al., 2019**). On the other hand, the main components of millet, such as phytochemicals, fibers and proteins, may account for its beneficial effects on diabetes and obesity (**Kam et al., 2016**). Where, millet proteins are mainly composed of four proteins, including prolamin, gluten, albumin, and globulin. Among these, prolamin is the major storage protein in millet (**Nishizawa et al., 2009**). Therefore, the present study was conducted to investigate the effects of feeding supplementing HFD with millet flour (MF) on some physiological functions in rats.

## 2. Materials and Methods:

**Materials:**

**Millet Grains:** Millet grains were classified and obtained from the National Center for Agricultural Research, Giza, Egypt.

**Rats:** Thirty-five of adult male rats (Sprague Dawley strain), weighing about 150±5 g were obtained from the Laboratory Animal Colony, Helwan, Egypt.

**Basal Diet Constituent:** Casein, cellulose, choline chloride, D-L methionine, vitamins and minerals constituents were purchased from El-Gomhoriya Pharmaceutical Company, Cairo, Egypt. Starch, soybean oil, and sucrose will be obtained from the Egyptian local market.

**Chemicals and Kits for Biochemical Analysis:** Kits for biochemical analysis and other chemicals were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

**Methods:**

**Preparation of Millet Flour:** Millet grains were cleaned and prepared before use according to the described methods by **Shobana et al., (2009)**. Briefly, the millet grains were moistened by water spraying, leaft for 10 min and pulverized. Then, it was sifted through a mesh stainless steel sieve (180mm openings) and the tailings were again pulverized and sifted through the same sieve. The process of pulverizing the tailings and sieving was repeated two more times and the millet endosperm flour fraction that passed through the first, second and third stages of sieving was pooled and termed as millet flour and packaged in an enclosed bag and stored in the refrigerator at 5 ºC till use.

**Preparation of Basal Normal (AIN 93M), and High Fat Diets:** All components of basal diet were mixed together to fulfil the desirable adequate dietary intake for keeping the health state of rats as confirmed by **Reeves et al., (1993)**. Concisely, each 1 kg diet consists of 140g casein (85% protein), 465.70 g corn-starch, 155 g dextrinized corn-starch, 40g soybean oil, 100g sucrose, 50g fiber, 10g vitamin mixture, 35g mineral mixture, 2.5g choline chloride, 1.8 g L-cysteine and 0.008g Tert-butylhydroquinone.

A high-fat diet (HFD) was prepared as described by (**Bhatt et al., 2006**). Briefly, the basal diet was supplied with 59% calories from fat based on lard and soybean oil, 21% calories from carbohydrate and 20% calories from protein. After that, the high-

fat diet was divided into four parts. The first part is a diet high in fat content only, while the second, the third and the fourth parts were supplemented with millet flour at levels of 5, 10 and 15%, respectively. Then diets are packaged in an enclosed bag and stored in the refrigerator at 5 ºC for use.

**Experimental Design**: All rats were housed at a room temperature of 25 ± 2 °C, relative humidity of 50–55% and 12 hr. light/12 hr. dark cycles in an animal house at the Faculty of Home Economics, Cairo, Egypt for one week for acclimatization. After the acclimatization period (one week), thirty-five adult male rats were divided into five main groups (7 rats each) as follows:

**Group 1**: Rats were fed on the normal basal diet and represented as normal rats (negative control group).

**Group 2:** Rats were fed on HFD and represented as a positive control group.

**Group 3:** Rats were fed on supplemented HFD with 5% millet flour (MF).

**Group 4**: Rats were fed on supplemented HFD with 10% of MF.

**Group 5**: Rats were fed on supplemented HFD with 15% of MF.

**Estimation of Feed Intake, Body Weight Gain, Percent Change of Body Weight gain and Feed Efficiency Ratio:** The daily feed intake (FI) of each rat was recorded and calculated based on the average daily intake during the experimental period (6 weeks). Body weight gain (BWG) was determined by the difference between the initial body weight of rats (IBW) and at the end of the experimental (FBW). The percent change of body weight gains and feed efficiency ratio (FER) were estimated using the following equations, respectively as described by **Kratochvílova et al., (2002)**:

$$\text{BWG (\%)} = \text{BWG}/\text{IBW} \times 100$$

$$\text{FER} = \text{BWG (g/d)}/\text{FI (g/d)}$$

**Blood Collection and Biochemical Assay:** At the end of the experimental period (6 weeks), all rats were fasted overnight (12 hr.), anaesthetized with diethyl ether and scarified. Blood samples were taken from the portal vein cava into dry clean centrifuge tubes and left at room temperature to clot and then centrifuged for 15 minutes at 4000 rpm for serum separation. Then, separated pure serum samples were carefully taken by an automatic pipette, drawn into the clean covered Eppendorf pipe, and kept at -20°C in a deep freeze until used for biochemical inspection.

**Biochemical Assay:**

**Estimation of Serum TL, TG, TC, LDL-c and HDL-c Levels and Atherogenic index:** Serum levels of total lipid (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) levels were estimated using commercial reagent kits (Biomed diagnostic, Egypt) and expressed as mg/dl according to the described methods by **Lutzke and Brauler (1990), Admundson and Zhou (1999)**, **Zhu et al., (2000)** and **and Young, (2001)**. Atherogenic index was calculated according to the formula adopted by (**Hostmark et al.,1995)** as follows:

$$\text{Atherogenic index} = (\text{TC-HDL-c})/\text{HDL-c}$$

**Estimation of Liver Functions:** Serum levels of aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) enzymes were measured calorimetrically using spectrophotometer adjusted at 505 nm according to the described method of **Young, (2001).** Serum alkaline phosphatase (ALP) activity was determined according to the method of **Young, (1997))** using spectrophotometer DU7400 adjusted at 510nm.

**Estimation of Kidney functions**: Serum concentrations of creatinine (Cr), urea nitrogen (BUN) and uric acid (UA) were measured according to the kit's instruction manual as mentioned by **Needleman et al., (1992)**, **Mitrovic et al., (2012)** and **Tietz et al., (2005)**, respectively.

**Estimation of Serum Glucose and Leptin Levels:** Blood glucose levels (BG) were determined immediately after serum separate using glucose enzymatic kit as described by **Siest et al., (1981).** Serum level of leptin hormone was determined using enzyme-linke dimmunosorbent (ELISA) assay according to the method of **Xiong et al., (2005)**.

**Estimation of Oxidative Stress Markers:** Serum lipid peroxide levels were estimated by the determination of the level of thiobarbituric acid reactive substances (TBARS) that were measured as MDA according to the described method by **Mihara and Uchiyama, (1978)** and expressed as nmol/ml. The activity of GSH enzyme was determined according to the disclosed method by **Beutler et al., (1963)** and expressed as nmol/ml. Activities of GPx enzyme were determined using the pyrogallol autoxidation methods as mentioned by **Yang et al., (1999),** respectively.

**Histopathological Examination:** The extent of high-fat diet on rats was evaluated by assessing the morphological changes in the liver and aorta sections. The histopathological screening process for the heart and aorta sections of all rats were carried out as referred procedures by **Bancroft and Gamble, (2002)**. Briefly, heart and aorta samples

were carefully washed in an isotonic solution, dried on a filter paper and immersed in buffer formalin (10%). Afterward, the fixed heart and aorta samples were dehydrated in graded ethyl alcohol from 50 to 100%. Subsequently, specimens were cleared by Xylol, immersed in paraffin bulk, sliced to 4-6 µm thickness and colored with Hematoxylin (HX) and eosin (E) for the inspection.

**Statistical analysis:** The obtained results were expressed as Mean ± SD. Data will be evaluated statistically with computerized SPSS package program (SPSS 22.00 software for Windows) using one-way analysis of variance (ANOVA). Significant difference among means will be estimated at $p<0.05$.

## 3. Results:

The results of the effect of the HFD diet-supplemented with MF at the different levels (5, 10 and 15%) on final body weight (FBW), body weight gain (BWG), feed intake (FI), BWG (%) and feed efficiency ratio (FER) in treated rats are recorded in Table **1**. The results found that the positive control group fed on HFD alone had a significant ($p<0.05$) increased in FBW, BWG, BWG (%) and FER, and decreased in feed intake, compared to the normal control group fed on normal basal diet. While, treating rats by feeding on HFD diet-supplemented with MF at the different levels results in a significant decrease in FBW, BWG and BWG (%), and FER, except treated rats with 5% of MF had no significant changes in FBW and FER, and increased in FI, compared to the positive control group. It was also noted that the improvement rates in FBW, and BWG augmented with rising the taken MF concentration.

Table 1: Effect of HFD diet-supplemented with MF on BWG, FI, BWG (%) and FE in treated rats

| Groups / Parameters | Negative group | Positive group | Treated rats with the MF at a level of: | | |
|---|---|---|---|---|---|
| | | | 5% | 10% | 15% |
| IBW (g) | 160.50±1.30 | 160.50±1.10 | 161 ± 1.79 | 160.75±1.11 | 160.5±1.22 |
| FBW (g) | 228.00±2.20$^{c}$ | 266.50±2.70$^{a}$ | 266.50±.12$^{a}$ | 240.50±1.44$^{b}$ | 226.50±1.80$^{d}$ |
| BWG (g) | 67.50±2.74$^{d}$ | 106.00± 2.33$^{a}$ | 105.50±3.54$^{b}$ | 79.75 ± 2.63$^{c}$ | 66.00±2.41$^{e}$ |
| FI (g/d) | 17.50±0.23$^{a}$ | 15.5±0.11$^{d}$ | 16.00±0.28$^{c}$ | 16.80± 0.16$^{b}$ | 16.8±0.14$^{b}$ |
| BWG (%) | 42.06±0.98$^{d}$ | 66.04±1.03$^{a}$ | 65.52±1.03$^{b}$ | 49.61±1.13$^{c}$ | 41.12±1.11$^{e}$ |
| FE | 0.09±0.03$^{c}$ | 0.15±0.02$^{a}$ | 0.15±0.04$^{a}$ | 0.11±0.02$^{b}$ | 0.09±0.04$^{c}$ |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same row are significantly different at $P < 0.05$

Results in **Table 2** demonstrate the effect of feeding on the HFD diet-supplemented with MF at the different levels (5, 10 and 15%) on the serum levels of total lipids (TL) triglycerides (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-c) and high density lipoprotein cholesterol (HDL-c) in rat-groups. In comparison to the negative control group, HFD induced a significant ($P < 0.05$) increase in serum concentrations of TL, TG, TC and LDL-c, and decreased serum HDL-c. However, in comparison to the positive control group, feeding on the HFD diet-supplemented with MF at the different levels (5, 10 and 15%) resulted in significantly ($P < 0.05$) lower in the serum levels of TL, TG, TC and LDL-c, and raise serum HDL-c. A better improvement in serum levels of lipid profiles was discovered with increasing levels of MF in treated rats.

Table 2: Effect of HFD diet-supplemented with MF on TL, TG, TC, LDL-c and HDL-c in treated rats.

| Parameters / Groups | | TL (mg/dl) | TG (mg/dl) | TC (mg/dl) | LDL-c (mg/dl) | HDL-c (mg/dl) |
|---|---|---|---|---|---|---|
| Control groups | Negative | 301.57±0.33$^{e}$ | 68.40±1.67$^{e}$ | 71.60±0.89$^{e}$ | 48.32±1.35$^{e}$ | 66.20±1.09$^{a}$ |
| | Positive | 396.43±0.61$^{a}$ | 126.2±1.09$^{a}$ | 132.40±1.52$^{a}$ | 75.60±0.55$^{a}$ | 46.40±0.55$^{e}$ |
| Treated rats with the MF at a level of: | 5% | 333.29±0.39$^{b}$ | 112.00±1.73$^{b}$ | 115.8±0.84$^{b}$ | 65.44±1.67$^{b}$ | 52.00±1.00$^{d}$ |
| | 10% | 322.86±0.36$^{c}$ | 90.60±2.97$^{c}$ | 99.40±1.34$^{c}$ | 58.44±1.18$^{c}$ | 58.00±1.00$^{c}$ |
| | 15% | 303.86±0.34$^{d}$ | 72.40±1.14$^{d}$ | 83.60±0.89$^{d}$ | 53.50±1.40$^{d}$ | 63.40±0.89$^{b}$ |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same column are significantly different at $P < 0.05$

**Figure 1** illustrated the effect of HFD alone and HFD-supplemented with the different levels (5, 10 and 15%) of MF on Atherogenic index (AI) in rats. The results revealed that rats fed on HFD alone have significant increase ($p<0.05$) in AI value (1.858±0.98), compared to that fed on normal basal

diet (0.081±0.82). In contrast, HFD-supplemented with 5, 10 and 15% of MF caused significant decrease in AI values (1.213±0.79, 0.714±0.77 and 0.320±0.82, respectively), compared to HFD alone. The better results in AI values were observed in rats treated with the highest level (15%) of MF, compared to the other two levels (5 and 10%).

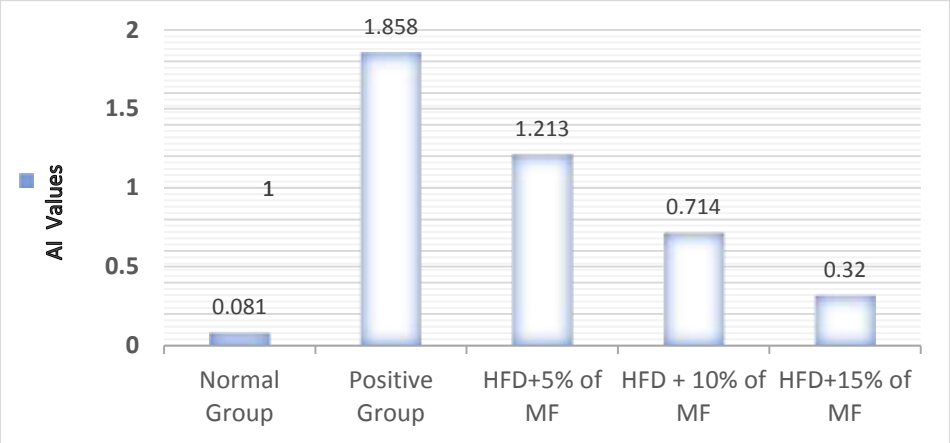



Figure 1: Effect of HFD diet-supplemented with MF on AI values in treated rats

The attained results in **Table 3** exhibit the effect of provision HFD without or with feeding on the complemented diet with FM on liver functions in rat-groups. Delimited results showed that the positive group feeding on the HFD alone had a significant (P<0.05) increment in activities of liver enzymes (AST, ALT and ALP), compared to a normal control group. However, combining a supplemented HFD by different levels of FM, results in a significant (p<0.05) lowering in the liver enzymes, compared with the positive control group. As exhibit, there is better improvement in liver function by the enhancement in the tested parameters with increasing levels of FM taken in the diet.

Table 3: Effect of HFD diet-supplemented with MF on serum activities of AST, ALT ALP enzymes in treated rats

| Parameters / Groups | | AST (U/L) | ALT (U/L) | ALP (U/L) |
|---|---|---|---|---|
| Control groups | Negative | 34.20±0.84$^{e}$ | 11.40±0.55$^{e}$ | 68.20±1.48$^{e}$ |
| | Positive | 59.60±0.55$^{a}$ | 25.20±0.45$^{a}$ | 116.80±0.84$^{a}$ |
| Treated rats with the MF at a level of: | 5% | 45.40±0.55$^{b}$ | 20.20±0.84$^{b}$ | 99.00 ±1.58$^{b}$ |
| | 10% | 41.40±0.55$^{c}$ | 16.00±0.71$^{c}$ | 89.60 ±1.52$^{c}$ |
| | 15% | 37.80±0.45$^{d}$ | 12.80±0.84$^{d}$ | 74.60±1.14$^{d}$ |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same column are significantly different at P < 0.05

The end outcome in **Table 4** shows that rats feeding on the HFD alone had a significant (p <0.05) increase in serum levels of creatinine (Cr), urea nitrogen (BUN) and uric acid (UA), as compared to that of the negative control rats. Feeding rats on the supplemented diet with MF at the three different levels had a significant (p<0.05) decrease in the serum Cr, BUN and UA levels, compared to feeding on HFD alone (positive group) showed a performance. The superior improvement in the serum levels of the tested variables was shown in the treated group with the highest level (15%) of MF.

Table 4: Effect of HFD diet-supplemented with MF on serum activities of Cr, BUN and UA in treated rats

| Parameters / Groups | BUN (mg/dl) | Cr (mg/dl) | UA (mg/dl) |
|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Control groups | Negative | 33.20±1.64[c] | 0.57±0.01 [d] | 2.72±0.25 [e] |
| | Positive | 42.00±1.22[a] | 0.70±0.01 [a] | 6.32±0.08 [a] |
| Treated rats with the MF at a level of: | 5% | 39.20±1.09[b] | 0.67±0.01 [b] | 4.52±0.47 [b] |
| | 10% | 37.00±0.70[b] | 0.63±0.00 [c] | 3.80±0.12 [c] |
| | 15% | 29.00±1.87[d] | 0.61±0.02 [c] | 3.24±0.15 [d] |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same column are significantly different at $P < 0.05$

As an appraisal of the effect of HFD on the levels of blood glucose and leptin hormone in treated rats, the results are recorded in **Table 5**. In comparison to normal rats fed on the normal basal diet, feeding rats on HFD induced a significant ($p<0.05$) increase in serum concentrations of blood glucose and leptin hormone. On the other hand, feeding rats on HFD-supplemented diet with the different levels of MF resulted in a significant amelioration in the serum levels of blood glucose and leptin hormone, compared with those feeding on HFD alone. The better results were observed in rats treated with the highest level (15%) of MF, compared to the other two levels (5 and 10%), as well as the positive control group.

Table 5: Effect of HFD diet-supplemented with MF on the levels of blood glucose and serum levels of leptin hormone in treated rats

| Parameters / Groups | | Blood Glucose (mg/dl) | Total Leptin (ng/ml) |
|---|---|---|---|
| Control groups | Negative | 78.80±1.64 [e] | 2.90±0.09 [d] |
| | Positive | 120.20±0.44 [a] | 9.74±0.18 [a] |
| Treated rats with the MF at a level of: | 5% | 97.40±1.95 [b] | 6.36±0.15 [b] |
| | 10% | 91.00±1.00 [c] | 4.58±0.19 [c] |
| | 15% | 85.00±2.00 [d] | 2.90±0.10 [d] |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same column are significantly different at $P < 0.05$

**Table 7** represents the effect of MF on the protection of rats from oxidative imbalance resulting from HFD. Serum levels of MDA and the activities of antioxidant enzymes (GSH and GPx) were used as indicators of this effect. The obtained results revealed that feeding on HFD alone induced a significant ($p<0.05$) rise in serum MDA level, and a lower in the activity of GSH and GPx enzymes, in comparison to feeding on the normal basal diet. Combining the different levels of MF with HFD significantly reduced serum levels of MDA and increased activity of the mentioned antioxidant enzymes, compared to HFD alone. Also, the results revealed that an increasing added level of MF increased added protection for treated rats as shown by the eminent amelioration of the tested parameters.

Table 6: Effect of HFD diet-supplemented with MF on serum levels of MDA and activities of GSH and GPx enzymes in treated rats

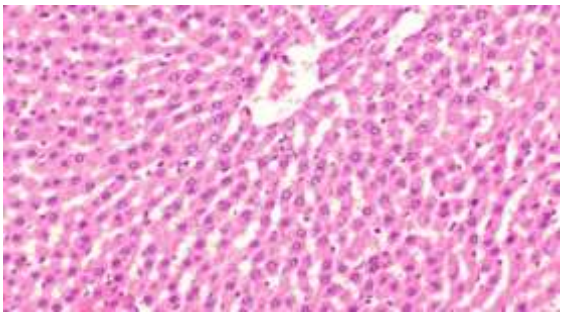
| Parameters / Groups | | GSH (mmol/dl) | GPx (mmol/dl) | MDA (mmol/L) |
|---|---|---|---|---|
| Control groups | Negative | 5.39±0.15[a] | 15.97±0.57[a] | 1.33±0.05[d] |
| | Positive | 4.23±0.16[e] | 11.21±0.13[e] | 2.33±0.21[a] |
| Treated rats with the MF at a level of: | 5% | 4.50±0.13[d] | 11.91±0.33[d] | 1.93±0.05[b] |
| | 10% | 4.75±0.04[c] | 12.65±0.15[c] | 1.65±0.03[c] |
| | 15% | 4.91±0.06[b] | 13.65±0.40[b] | 1.44±0.01[d] |

Data are expressed as the mean ± SD; Mean values with different superscript letters at the same column are significantly different at $P < 0.05$

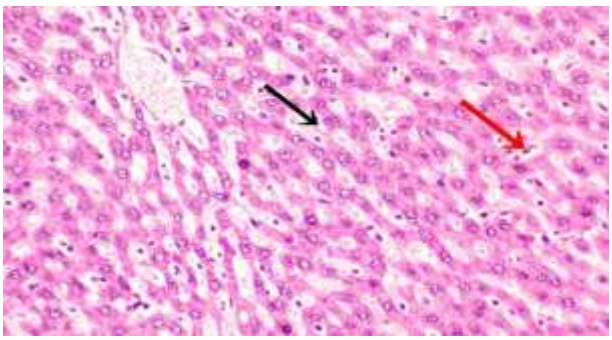
Microscopic investigation of liver sections from the negative control group (normal rats) discovered a normal histological arrangement without any pathological amendment (**Picture 1**). On contrary, liver sections from rats fed on HFD alone (positive rats) have hepatocellular vacuolar degeneration and Kupffer cells activation (**Picture 2**), as well as hepatocellular necrosis associated with inflammatory cells infiltration and portal infiltration (**Picture 3**). Meanwhile, liver sections from rats fed on HFD-supplemented with 5% of MF showed slight kupffer cells activation and inflammatory cells infiltration in the portal triad as shown in **Picture 4**. In contrast, some liver sections

from rats fed on HFD-supplemented with 10% of MF revealed slight kupffer cells activation and few inflammatory cells infiltration in the portal triad (**Picture 5**), whereas other sections exhibited no histopathological alterations (**Picture 6**). Furthermore, liver sections from rats fed on HFD-supplemented with 15% of MF revealed no histopathological alterations (**Picture 7**) except slight kupffer cells activation in some sections (**Picture 8**).

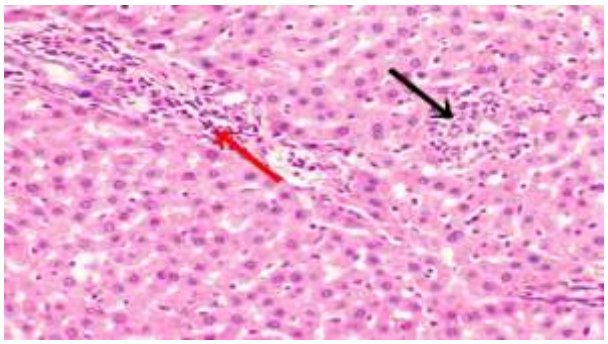
Microscopically, aorta sections from normal rats revealed normal histological structure as shown in **Picture 9**. On contrary, aorta sections from positive rats fed on HFD alone showed marked vacuolization in the wall as shown in **Picture 10**. On the other hand, aorta sections from treated rats fed on HFD-supplemented with 5 and 10% of MF showed progressive vacuolization in the tunica media from moderate in some sections to slight in other sections as shown in **Pictures 11 and 12**, respectively. However, aorta sections from rats fed on HFD-supplemented with 15% of MF described no histopathological alterations in some investigated sections (**Picture 13**) and slight vacuolization in the tunica media in other sections (**Picture 14**).



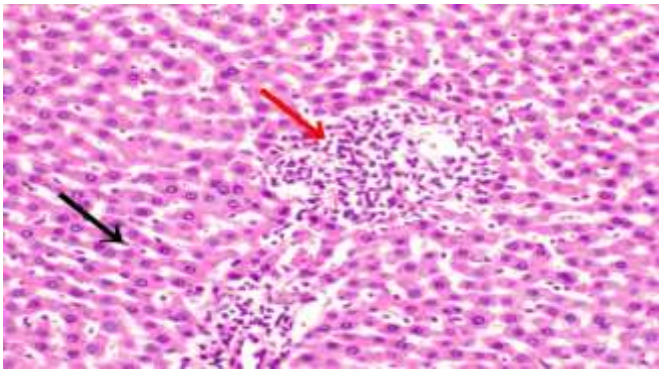
ıotomicrograph of liver sections from normal rats showing the normal histological arrangement of hepatic tissue (H & E X 400).



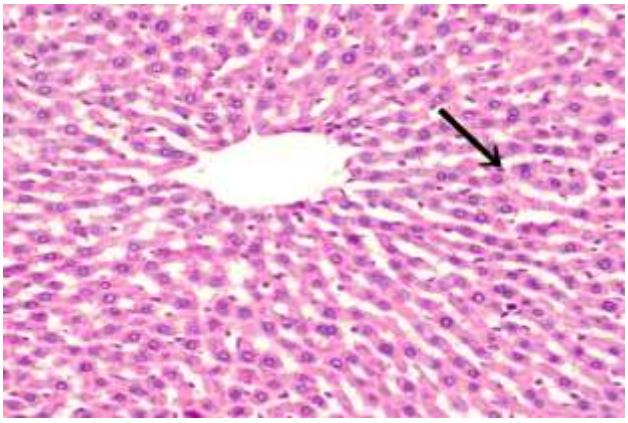
Picture 2: Photomicrograph of liver sections from positive group showing hepatocellular vacuolar degeneration (black arrow) and Kupffer cells activation (red arrow) (H & E X 400).



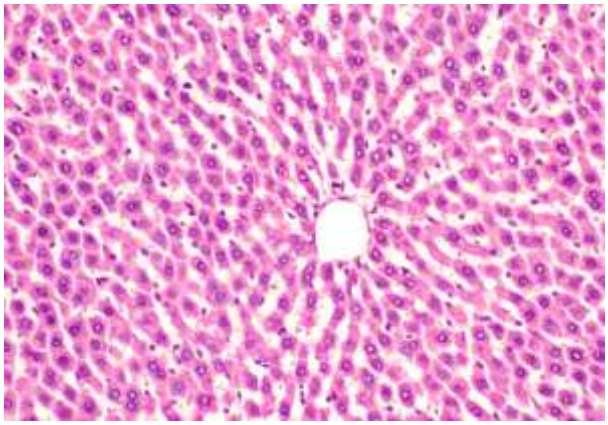
Picture 3: Photomicrograph of liver sections from positive group hepatocellular necrosis associated with inflammatory cells infiltration (black arrow) and portal infiltration with inflammatory cells (red arrow) (H & E X 400).



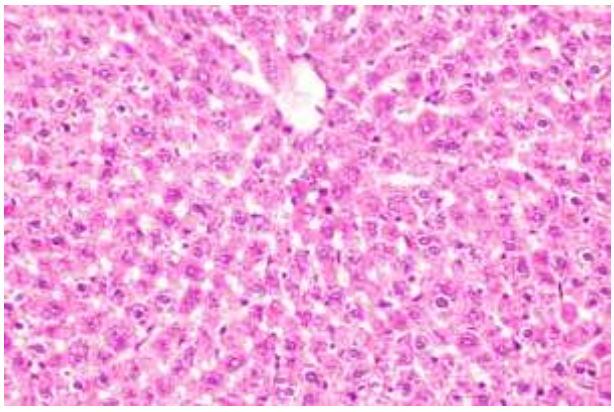
Picture 4: Photomicrograph of liver sections from rats fed on HFD- supplemented with 5% of MF showing slight Kupffer cells activation (black arrow) and inflammatory cells infiltration in the portal triad (H & E X 400).



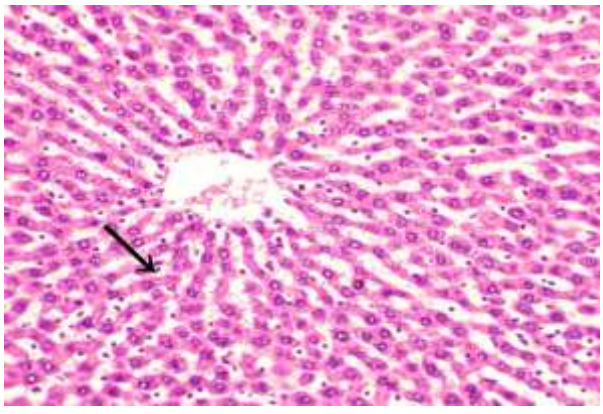
Picture 5: Photomicrograph of liver sections from rats fed on HFD-supplemented with 10% of MF showing slight Kupffer cells activation (H & E X 400).



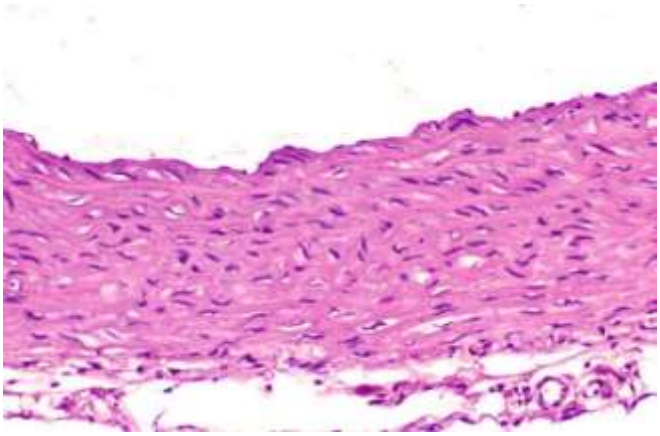
Picture 6: Photomicrograph of liver sections from rats fed on HFD-supplemented with 10% of MF showing no histopathological alterations (H & E X 400).



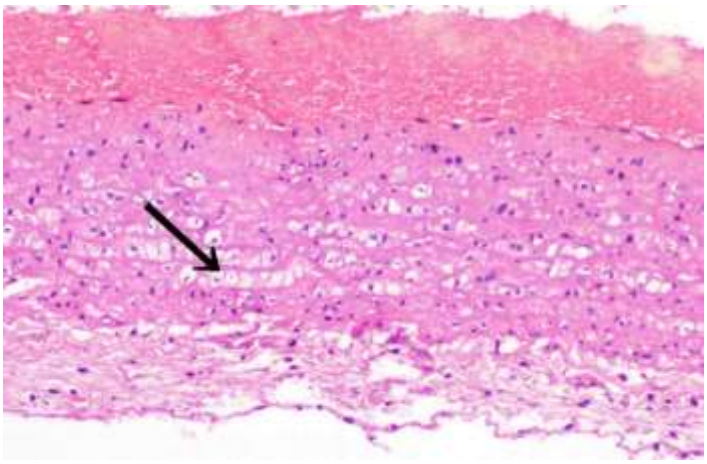
Picture 7**:** Photomicrograph of liver sections from rats fed on HFD-supplemented with 15% of MF showing normal histoarchitecture of hepatic tissue (H & E X 400).



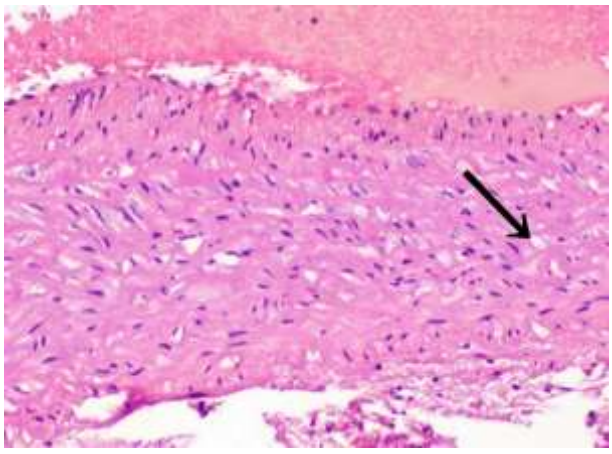
Picture 8: Photomicrograph of liver sections from rats fed on HFD-supplemented with 15% of MF showing slight Kupffer cells activation (H & E X 400).



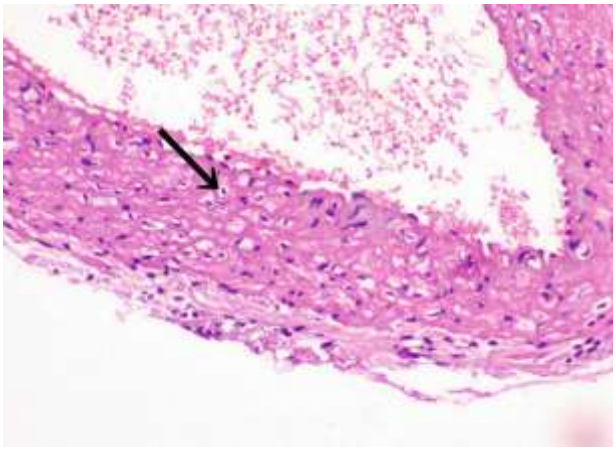
omicrograph of aorta sections from normal rats showing normal histological structure (H & E X 400).



Picture 10: Photomicrograph of aorta sections from positive group showing vacuolization in the wall (black arrow) (H & E X 400).

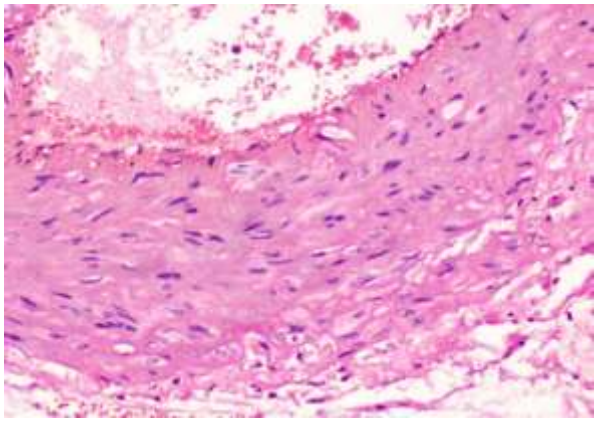


Picture 11: Photomicrograph of aorta sections from



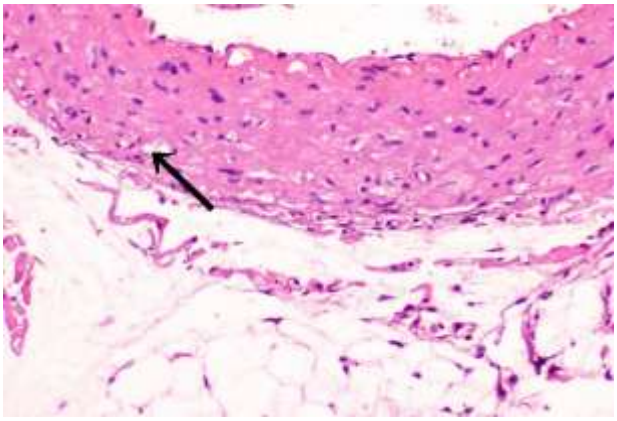
Picture 12: Photomicrograph of aorta sections from

rats fed on HFD-supplemented with 5 and 10% of MF showing slight vacuolization in the tunica media (H & E X 400).



Picture 13: Photomicrograph of aorta sections from rats fed on HFD-supplemented with 15% of MF showing no histopathological alterations(H & E X 400).

rats fed on HFD-supplemented with 5 and 10% of MF showing moderate vacuolization in the tunica media (H & E X 400).



Picture 14: Photomicrograph of aorta sections from rats fed on HFD-supplemented with 15% of MF showing slight vacuolization in the tunica media (H & E X 400).

## 4. Discussions

The present study was conducted to investigate the effects of feeding supplementing HFD with millet flour (MF) on some physiological functions in rats. To achieve the goals of the study, serum levels of lipid profile, blood glucose, and leptin hormone, and atherogenic index, functions of liver and kidney as well as oxidative stress were evaluated in rats. In addition to histopathological investigation of the liver and aorta.

High-fat diets have been considered as the most popular model among researchers for inducing obesity in animals due to its high similarity in mimicking the usual route of obesity episodes in humans (**Buettner et al., 2005**). The present result has revealed that rats fed on a high-fat diet for 4 weeks have a significant increase in final body weight (FBW), body weight gain (BWG), percent of BWG and feed efficiency ratio (FER) and a decrease in a food intake, compared to that of rats fed on normal basal diet.

Hyperphagia may be an important reason, although high energy density, palatability and other metabolic effects may also contribute to this correlation. Also, (**Hill et al.,2000**) demonstrated that high-fat diet diets containing ≥ 30% of total energy from fats induce obesity in rats and mice as a result of increased energy intake and efficient energy storage. According to **Kusnoki et al., (2000**), a high fat diet is an important factor in the development of obesity, leading to accumulation of body fat even in the absence of an increase in caloric intake in fed rats with a high fat diet. Recently, several researchers indicated that a high-fat diet is harmful, being high in energy and therefore leading to overweight or obesity than rats fed a normal diet (**Macfarlane et al., 2012 and Haghshenas et al., 2014**). The present results were in accordance with (Rezq, 2017) who recorded that rats fed on HFCD had a significant increase in body weight, compared to normal rats fed on the normal basal diet. Additionally, **Ogungbemi et al., (2017)** reported that a high-fat diet caused a significant increase in FBW and FER. Also, **Abdulrahman et al., (2020)** showed significantly higher in FBW of rats fed on an HFD. Recently, **Ibrahim et al., (2022)** reported that obese rats fed HFD have significant ($P<0.05$) increase in FBW, BWG and RWG, compared to that of the fed rats on normal basal diet.

High-fat diet supplemented with MF at level of 5%, 10% and 15% conversely caused a remarkable reduction of FBW, BWG, BWG%, FI and FER compared to the high-fat diet alone. The reduction in food consumed and body weight are more pronounced with increasing levels of Millet. The present result suggested millet can prevent body weight gain. This result was in accordance with the reported results by **Sarma et al., (2018)** who revealed that arabinoxylan (a polysaccharide) presented in millet has the ability to prevent adiposity in high fat diet-induced rats. Additionally, **Slavin, (2005**) mentioned that millets are a rich source of micronutrients, gluten-free, low glycemic index, fiber, and possess antioxidant properties. Millets play a vital role in controlling obesity rates, regulating fat percentage in the body, helping in reducing adipose tissue in the body and promoting weight loss. Also, epidemiological studies support

the fact that dietary fiber intake strongly prevents obesity and is inversely associated with body fat and body mass index at all levels of fat intake.

High-fiber foods have much less energy density compared with high-fat diets and can displace energy. Eating an equal weight of high-fiber food increases satiety. The bulking and viscosity properties of dietary fiber are mainly responsible for influencing satiety (**Burton-Freeman 2000**).

Dyslipidemia is another important lineament in the manner of development of obesity which is characterized by hyperlipidemia, hypertriglyceridemia with increased levels of LDL-c and VLDL-c (**Klop et al., 2013**). Chronic dyslipidemia has been characterized as a major risk factor for cardiovascular disease and atherosclerosis (**Mbikay 2012**). In the present study, high-fat diet exposure resulted in a significant increase in serum TL, TG, TC, LDL-c levels and atherogenic index to decrease serum HDL-c level. In addition, histopathological results showed that consumption of a high-fat diet may play a crucial role in the pathogenesis of vocalizations in the wall and perivasculitis with dilated and congested blood vessels in the aorta. The present results were in accordance with (**Rezq et al., 2017 and Ibrahim et al., 2022**) who showed that high-fat diet results in dyslipidaemic changes by increasing serum levels of TG, TL, TC and LDL-c and decrease serum HDL-c levels. Additionally, (**Puskas et al., 2004**) demonstrated that the intracellular lipid accumulation in cardiomyocytes is in response to high fat diet. Excess lipid and cholesterol in the bloodstream can form plaque in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart and resulting in a heart attack. When there is too much LDL-c in the blood, it is deposited in the blood vessels, where it can build up to hard deposits and cause atherosclerosis (**Ma, 2004**). Further, atherogenic index is regarded as a marker for various cardiovascular disorders; the higher the value, the higher the risk of developing cardiovascular disease and vice versa (**Takasaki, 2005**). The dyslipidemia in rats fed a high-fat diet may be attributed to the activity of lipoprotein lipase, which is augmented in hypercholesterolemic animals. Lipase transforms VLDL-c to LDL-c, and leads to an increased serum concentration of LDL-c. The uptake of LDL-c depends on receptors in the plasmatic membrane, and these are reduced in number when the cell has enough cholesterol. Further, the alteration of lipid profile induced might be the activation of intestinal fat absorption and lipolysis (**Saravanan et al., 2012**).

In the present study, supplementation of high-fat diet with the of MF induce significant attenuate in serum TL, TG, TC, and LDL-c levels, and atherogenic index and increased serum HDL-c levels in rats. The increase in serum level of HDL-c and the decrease in atherogenic index were found to be in a dose dependent manner; that is, supplementation with MF at a dose of 15% shows a better effect in comparison to 5% and 10%. Thus, it can be concluded that MF possess antihyperlipidemic and cardioprotective potential.

The high fiber present in millets plays a major role in cholesterol lowering eliminating LDL from the system and increasing the effects of HDL. In a study, rats fed with diet of treated starch from barnyard millet had shown to lower blood glucose, serum cholesterol and triglycerides (**Kumari et al.,1997**). Improved plasma levels of Nutritional and Health Benefits of Millets, high density lipoprotein (HDL) cholesterol in genetically obese type -2 diabetic mice under high fat conditions were observed on feeding millet (**Park et al, 2008**).

Several studies have the millets have also shown to lower significantly the concentrations of serum triglycerides. millet may prevent cardiovascular disease by reducing plasma triglycerides in hyperlipidemic rats (**Lee et al., 2010**). Rats fed with diet of treated starch from millet had shown to lower blood glucose, serum cholesterol and triglycerides (**Kumari et al.,1997**).

Additionally, Obesity is an inflammatory disorder, Inflammation results in free radical production. Antioxidants from foods play a crucial role in scavenging the free radicals and reducing inflammation**.** An article published in the American Chemical Society showed that millets showed to have a good number of phenolic compounds and possess antioxidant, metal chelating and reducing powers **(Chandrasekara et al., 2010).** A study on flavonoids and phenolic compounds in millet revealed that millet is a repository of antioxidants, flavonoids, and phenolic acids that have free radical scavenging capacity there helps to treat oxidative stress-related disorders like obesity, cardiovascular diseases **(Nambiar et al., 2012).**

Insulin resistance in humans can be linked to lifestyle and can be notice more as a cause of lipid deposition in a caloric excess **(Unger et al., 2010).** Thus, excessive caloric intake can lead to hyperinsulinemia, which raises sterol regulatory element-binding protein- 1c expression in beta cells, resulting in increased lipogenesis and obesity **(Muhlhausler et al., 2009).** Insulin resistance is associated with a number of metabolic disorders such as obesity, hyperlipidemia, and hypertension. High-fat diet intakes were shown to contribute to syndromes such as hyperlipidemia, glucose intolerance, hypertension, and atherosclerosis **(Sumiyoshi et al., 2006).**

Numerous evidence indicated that in experimental animals, high-fat diets resulted in

disturbance in glucose metabolism and impaired glucose tolerance **(Vessby, 2000 and Lichtenstein et al., 2000).** The present results showed significant increase of serum glucose level and decrease of serum insulin level in rats fed on high-fat diet, compared to that fed on normal basal diet. This result was agreed with **(Kusunoki et al., 2000)** who showed hyperglycaemia, dyslipidaemia and hyperinsulinaemia in rodents fed a high-fat diet. **(Srinivasan et al., 2004)** revealed that the feeding on high-fat diet for a period of 30 days presents hyperglycemia as shown by increased levels of serum glucose, insulin and insulin resistance. **(Huang et al., 2004)** found that feeding high-fat diet results in decrease of insulin secretion (hypoinsulenemia). Some previous studies revealed that hyperinsulinemia and insulin resistance are common features of obesity in humans **(Kay et al.,2001)** and experimental animals **(Amin et al., 2009).** Recently, **(Abo- Raya et al.,2013)** showed that fed rats on high fat-diet for 6 weeks had significant lower in serum insulin level than those fed on basal diet. Also, **(Saravanan et al., 2014)** showed significant increase in body weight and serum glucose, lipid profile and decrease of serum insulin levels in high-fat diet rats.

Millet is lower on the glycemic index (GI) than many other grains. That means it raises your blood sugar slowly and gradually instead of in quick spikes. High-fiber, low-GI foods keep blood sugar steady, lower cholesterol, and help you lose weight. All these things are helpful for people with diabetes. In contrast, the present results showed reduction of serum glucose and higher in insulin levels in treated obese-hyperlipidemic rats with millet. These results are consistent with those of previous studies which revealed millet has improved antioxidant status and controlled blood sugar levels **(Rajasekaran et al., 2004).** millet has been reported to be beneficial for type 2 diabetics **(Ugare et al., 2011)**.

A group of grains called millets, may help lower the risk of developing type 2 diabetes and lower A1C, or average blood sugar over about three months, in individuals managing diabetes, according to a study published in That's because these grains have a lower glycemic index than alternatives such as white rice and refined wheat, the study authors note **(Anitha et al., 2021)**. Also, **Almaski et al., (2019)** concluded that millets may reduce both fasting and post prandial (after meal) blood sugar levels in healthy individuals as well as for type 2 diabetes.

Adipocytes secrete a variety of peptide hormones called adipocytokines such as leptin, adiponectin, visfatin, resistin, tumour necrosis factor-$\alpha$ and interleukin-6, which play a role in energy regulation **(Garg, 2006)**. Leptin is a common protein produced by the adipose tissue and highly correlates with body fat, suggesting that obese persons are insensitive to endogenous leptin production. It is a key fat-derived regulator of food intake and energy expenditure, and its secretion levels are usually positively correlated with the extent of the triglyceride stores in adipocytes **(Staiger et al., 2005)**. In the present study, result showed that serum leptin level increased significantly in the high-fat diet control group compared with the normal control group. Substitution of dietary carbohydrate for fat has been shown to increase plasma leptin **(Weigle et al., 2003)**. The present experimental diet consisted of more fat, and this might have accounted for the elevated levels of leptin, consistent with literature reports **(Handjieva-Darlenska et al., 2009).**
**Huang et al., 2004, Abo-Raya et al., 2013 and Saravanan et al., 2014)** showed that rats fed on high fat-diet had high serum leptin hormone level when compared with those fed on normal basal diet. Treated obese-hyperlipidemic rats with millet results in significant decrease of serum leptin level compared to untreated obese-hyperlipidemic rats. The decrease in plasma leptin concentration has been reported following energy restriction **(Dubuc et al., 1998)**. These observations suggested that the decrease of serum leptin levels after millet supplementation may be attributable to their effect on the decrease of food intake and body weight and consequently the decrease of lipid accumulation in the adipocytes.

The present study provides a perfect correlation between serum lipid peroxidation products as indicator by level of GSH and activity of antioxidant enzymes which play an important role in the antioxidant system. It showed that fed rats on high-fat diet induced significant increase of serum decrease serum GSH level and activities of GPx enzyme, compared to that fed on normal basal diet. The decrease in serum activity of antioxidant enzymes, as seen in serum of obese-hyperlipidemic rats, can lead to the excessive availability of superoxide and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products. High-fat diets result in the release of free fatty acids by the action of lipoprotein lipase with increase serum triglycerides and cause lipotoxicity, which results in insulin receptor dysfunction. The release of excessive free fatty acids provokes lipotoxicity, as lipids and their metabolites create oxidative stress **(Zhang et al., 2007).**

The present result was agreed with **(Amirkhizi et al., 2007)** who showed that increase the production of reactive oxygen species as well as reduced antioxidant defense mechanisms have been suggested to play a role in both humans and animal models of obesity. Further, lipid alterations have

been considered as contributory factors to oxidative stress in obesity **(Leopold et al., 2008).** Hypertriglyceridemia results in obese rats participate in the alteration of oxidant-antioxidant balance, suggesting increase the bioavailability of free fatty acids and lipid peroxidation **(Amirkhizi et al., 2007)**. Hyperlipidemia induces oxidative stress and increase lipid peroxidation **(Moussa, 2008).** Recently, **(Denisenko et al., 2013)** showed that fed animals on high fat diet inhibits activity of blood antioxidant enzymes and elevate lipid peroxidation (MDA).

In contrast, feeding rats on high-fat diet enriched with the different levels of both millets significantly ameliorate antioxidant system in rats as showed by decreasd serum MDA level and increased GSH level and elevate serum activity of antioxidant enzymes.

The millet can normalize the elevated lipid peroxidation and improved susceptibility to oxidative stress associated with depletion of antioxidants in obese-hyperlipidemic rats. The antioxidant properties of millet might be attributed to its content of phenolic and flavonoid compounds.

Phenolic compounds are efficient scavenger of free radicals as well as transition metal ion chelating agents. Flavonoids possess a chemical structure with hydroxyl position in the molecule that is involved in proton donating and radical scavenging mechanism **(Hou et al., 2003)**. Flavonoids, phenolic acids and tannins can terminate oxidative chain reactions by eliminating free radical intermediaries and inhibiting other oxidation reactions **(Jeon et al., 2012)**.

## 5. Conclusion

From this study, it may be concluded that millet flour has an antiobesity effect, antihyperlipidemia, cardioprotective capability, hypoglycaemic and ameliorate the antioxidant defences in rats fed on a diet high in fat. Therefore, it may be used in complementary medicine to treat obesity, hyperlipidaemias and diabetes. So, regular intake of millet flour or using it in food products may benefit in enhancing functional foods with the improvement of health status.

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