Ameliorative effect of capsaicin against cardiac dysfunction induced by high fat diet in adult male rat

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SUMMARY

Overconsumption of high fat diet is a leading cause for developing obesity, which is strongly related to cardiovascular diseases. To elucidate the cardiac dysfunction induced by the effect of high fat diet and to evaluate the potential ameliorative effect of capsaicin against this toxicity, 40 adult male rats were divided into four groups: High fat diet (HFD) group contained 10 rats that were fed on HFD for 8 weeks. High fat diet and capsaicin group (HFD+CAP) group contained 10 rats that were fed on HFD + CAP for 8 weeks besides control groups. The extent of cardiac toxicity was evaluated physiologically and biochemically in addition to homogenate and histological examination of cardiac tissue. High fat diet led to significant increase in body weight, heart weight and body mass index. Moreover, HFD group showed significant increase in heart rate, systolic blood pressure with decrease in EF%. Biochemical investigation revealed significant increase in serum glucose, insulin level with insulin resistance, besides increase in serum total cholesterol, triglycerides and free fatty acids. HFD heart homogenate revealed decrease in total energy charge and total antioxidant capacity. Furthermore, histological examination displayed alteration of the normal histological structure of the heart tissue, increase in collagen deposition and significant increase in the immunopositivity of the inflammatory marker COX-2 and the apoptotic marker caspase-3. These effects were partially alleviated by consumption of capsaicin parallel with high fat diet in (HFD+CAP) group. However, even if capsaicin could ameliorate the effects of high fat diet, it is better to avoid HFD, as it can induce cardiac dysfunction.

Key words: Capsaicin – High fat diet – Obesity – Cardiac dysfunction – Inflammation – Apoptosis

INTRODUCTION

Consumption of a high-energy diet boosts a positive energy state and induces development of overweight and obesity (Swinburn and Egger, 2002). Obesity remains a global public health problem related to health behaviours and health outcomes with increased prevalence globally (Arroyo-Johnson and Mincey, 2016). Obesity is strongly related to cardiovascular diseases such as coronary heart disease (CHD) and heart failure (HF) (Stanley et al., 2012). Oxidative stress characterizes a recognized feature of obesity and is concerned in both vascular dysfunction

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and cardiac fibrosis (Aubin et al., 2008; Lu et al., 2008). Many studies displayed that obesity's experimental model developed hypertension, hypercholesterolemia, hyperinsulinemia, renin–angiotensin system activation, and increased renal oxidative stress (Carroll et al., 2006).

The adult heart gains most of its energy from the oxidation of fatty acids (FAs), of which there are high levels in patients who feed on a high-fat diet (HFD) (Lopaschuk et al., 2010; Rider et al., 2013). Even though FAs are the main energy source of the myocardium, long-term exposure to free FAs may lead to fat deposition in cardiomyocytes and around the heart (Ji et al., 2017; Hu and Zhang, 2017). The harmful consequence of prolonged exposure to extra fats in the circulation on the heart is a result of the accumulation of toxic metabolic derivatives, which can lead to myocyte dysfunction and death via beginning of certain signalling cascades (Ji et al., 2017; Hu and Zhang, 2017)**.** Echocardiography is an ultrasound-based imaging method that enables serial, in vivo structural and functional characterization of the heart. Transthoracic echocardiography (TTE) has been widely used as a first-line investigation for the diagnosis of numerous cardiac disorders, including cardiomyopathies, valvular abnormalities and congenital heart defects. The ventricular ejection fraction (EF), the ratio of the stroke volume over the end-diastolic volume expressed as a percentage, is frequently used to gain an overall assessment of ventricular systolic function in humans and in small animals (Wang et al., 2018).

On the other hand, the co-administration of a natural antioxidant would counteract the deleterious effect of a high-fat diet on endothelial-mediated relaxation and reactive fibrosis (Baur and Sinclair, 2006; Das and Maulik, 2006). In consequence, the effect of HFD to develop an obesity state can be prevented by several factors like capsaicin (8-methyl-N-vanillyl-6-nonenamide), which is a compound with spicy smell and is the main capsaicinoid in chili peppers. It has a molecular formula ($C_{18}H_{27}NO_3$). Capsaicin shows monoclinic rectangular flaky colourless crystals (Pi et al., 2017). Capsaicin has been widely used in clinical practice. In cardiovascular studies, capsaicin was found to prevent obesity, induce the apoptosis of cancer cells, lower blood pressure and reduce blood lipids (Wong and Gavva, 2009). Capsaicin may show an anti-inflammatory effect via regulating the expression of some pro-inflammatory cytokines (such as COX-2) (Hwang et al., 2009). According to Woods et al., administration of a high-fat diet is an experimental model reproduces many features of human obesity (Woods et al., 2003). Therefore, our study aimed to achieve a new vision to the cardiac dysfunction effect of high fat diet by investigation of the hemodynamic, histopathological and biochemical changes in heart tissue in adult male rats, and to evaluate the potential ameliorative effect of capsaicin against this toxicity.

MATERIALS AND METHODS

Animals

40 healthy adult male rats (6-8weeks) weighing 180-220 g were used in this study. Rats were obtained from the Animal House of the Faculty of Medicine, Zagazig University, Egypt. They were housed in a temperature- and light-controlled room (12 h light/dark cycles) with free access to food and water. All experiments were performed in accordance with relevant guidelines and regulations of the Institutional Animal Care and Use Committee, Zagazig University (ZU-IACUC committee), approval number *ZU-IACUC/3/F/85/2020.*

Diet preparations

The high fat diet consisted of 42% lipids, 36% carbohydrates, and 22% proteins (kcal), the normal diet (ND) consisted of 12.5% lipids, 63.2% carbohydrates, and 24.3% proteins (kcal). To assess the therapeutic benefit of Capsaicin (0.003% of CAP, which correspond to a 0.399 mg/kg body weight) was added directly to rat chow (Baskaran et al., 2019).

Experiment protocol

40 adult male rats were divided into four groups, each containing 10 rats: Control group, which subdivided into normal diet (ND) subgroup, which contained 10 rats that were fed on normal diet (ND) for 8 weeks, and Capsaicin (CAP) subgroup,

which contained 10 rats that were fed on ND supplemented by Capsaicin for 8 weeks. High fat diet (HFD) group contained 10 rats that were fed on HFD for 8 weeks. High fat diet and capsaicin group (HFD+CAP) group contained 10 rats that were fed on HFD + CAP for 8 weeks. Female rats were excluded to avoid hormonal changes that might occur during the estrus cycles. The extent of cardiac toxicity was evaluated physiologically beside biochemical and histological examination of cardiac tissue samples from sacrificed rats.

Anthropometric measures

Measuring body weight: the animals were put in closed plastic containers and were weighed a day before the experiment and at the last day. The results were written in a record for each labeled rat (Brennan et al., 2009). Measuring rat length: nose-to-anus length was measured at the start and at the end of the experiment. An assistant was holding rats from the tail to lengthen the body, ensure the real nose-to-anus length of the animal and avoid false measures. A metal ruler graduated in centimeters was used by holding zero end at the anus and record the reading that reached the nose (Novelli et al., 2007). Calculating BMI index: body mass index (BMI) equals body weight (gm) / length^2 (cm^2), this index can be used as an indicator of obesity where the cutoff value of obesity BMI is more than 0.68 gm/cm^2 (Novelli et al., 2007).

Measurement of hemodynamic parameters

At the conclusion of the experiments, the heart rate, systolic blood pressure (SBP) was assessed by using the noninvasive tail-cuff method with a Narco BioSystems Electro-Sphygmomanometer. The average of two readings was recorded for each measurement.

Echocardiography

At week 8, rats were anaesthetized using 3% pentobarbital sodium (0.1 mL/100 mg) and their cardiac function and ventricular dimensions were evaluated by transthoracic echocardiography using a GE vivid E9 equipped with a 13-MHz phased-array transducer. All rats' hearts were recorded at the level of the papillary muscle in 2D and M-mode.

The parameters of left ventricular end diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD) were measured. Functions were assessed by the following parameters: endocardial fractional shortening (FS), and ejection fraction (EF). left ventricular percent fractional shortening, (FS) was calculated from the M-mode using the following equation FS (%): [(LVIDD - LVISD)/LVIDD] X 100%. Left ventricular ejection fraction (EF%) was automatically calculated by the echocardiography machine according to the Teicholz formula (Platt et al., 2017). The parameters were determined for three cardiac cycles and averaged.

Biochemical study

Blood samples (about 8 ml/rat) were obtained at the end of the experimental period after overnight fasting and measurement of blood pressure (between 9:00-11:00 a.m.), blood samples were obtained from retroorbital venous plexus of each rat after ether inhalation (Sharma et al., 2014). The blood samples were allowed to clot at room temperature before centrifuging at 3000 rpm for 15 minutes. The serum was stored at -20° C.

Serum Biochemical analysis: Serum glucose level: by using glucose enzymatic (GOD-PAP)-liquizyme Kits (Biotechnology, Egypt). Serum insulin level: by using rat insulin enzyme-linked immunosorbent assay kit BioSource (Biotechnology, Egypt).

Calculation of homeostasis model assessment of insulin resistance (HOMA-IR): The following equation was used; [insulin (µU/mL) x glucose (mg/dl) /405] (Mari et al., 2005; Gutch et al., 2015). Healthy Range: 1.0 (0.5-1.4), Above 2.5 indicates insulin resistance.

Total cholesterol (TC), free fatty acids (FFA) and triglycerides (TG) were measured from serum according to colorimetric kits from Biovision (Egypt) and the manufacturers' protocol. Concisely, serum sample was matched with assay buffer and enzyme mixture, incubated in dark and measured at 570 nm utilizing a plate reader. Quantification was done from standard curve using the corresponding standards for cholesterol, TG, or FFA.

Cardiac dissection

After 8 weeks of feeding, the hearts were rapidly excised from the decapitated rats. The heart weight, and the ratio heart/body weight of each rat were measured.

Homogenate heart tissue analysis

Heart samples were homogenized in 50 mM phosphate buffer (pH 7.4) and sonicated over ice. The sonicated homogenates were centrifuged for 20 min at 3,200 × g at 5°C. A supernatant portion was dialyzed for 24 hours against two changes of cold 50 mM phosphate buffer.

Adenine nucleotide analysis: the supernatant of homogenized heart tissue was neutralized with an equal volume of 1M Na_2HPO_4 and centrifuged again at 10,000g and 4°C for 10 minutes. The supernatant was filtered through a 0.22 µm filter. Next, 50 µl aliquots were analysed using a high-performance liquid chromatography (HPLC) method with a Beckman C18 column (5 µm, 250 × 4.6mm). Analytes were isocratically eluted using 96% 0.05M KH2PO4 (pH 6.5) and 4% methanol for 30 minutes. Concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) were determined at 254 nm using an external standard method for quantification. Total energy charge was defined as (ATP + ADP/2)/ (ATP + ADP + AMP) (Chen et al., 2018).

Total Antioxidant Capacity (TAC) was measured according to Cell Biolabs' OxiSelect™ Total Antioxidant Capacity (TAC) Assay Kit, based on the reduction of copper (II) to copper (I) by antioxidants such as uric acid. Upon reduction, the copper (I) ion further reacts with a coupling chromogenic reagent that produces a color with a maximum absorbance at 490 nm. The net absorbance values of antioxidants are compared with a known uric acid standard curve. Absorbance values are proportional to the sample's total reductive capacity. Results are expressed as "ng/mg".

Histological and morphometric examination

LV transverse sections of seven animals from each group were fixed in 10% buffered formalin and embedded in paraffin. Thick sections of 1 µm were cut from the tissue block and stained with haematoxylin and eosin, and with the collagen specific stain Masson's trichrome staining. Phosphate buffer (PBS), dissection set, 10% formal saline, alcohol, xylene and paraffin wax for preparation to light microscopic examination. H&E and Masson's trichrome stains were purchased from faculty of medicine, Zagazig University.

The myocyte cross-sectional area was determined for at least 100 myocytes per haematoxylin and eosin stained slide. The myocyte cross-sectional area measurements were obtained from digitized images (40× magnification lens) at Anatomy Department, Faculty of Medicine, Zagazig University. Myocyte cross-sectional area was measured using a digitizing pad, and the selected cells were cut transversely with the nucleus clearly identified in the centre of the myocyte. Area % collagen fibre was determined for the entire Masson's trichrome stained cardiac section were obtained from digitized images (40× magnification lens) at Anatomy Department, Faculty of Medicine, Zagazig University. The components of the cardiac tissue were identified according to color level as follows: green for collagen fibers; blue for myocytes; and white for interstitial space.

Immunohistochemical study for COX-2 and caspase-3 in the heart tissue

COX-2 antibodies, Caspase-3 antibodies, and Kits for enzymes assay were purchased from (Sigma-Aldrich chemical company, Germany and purchased from Sigma-Egypt). The immunohistochemical staining of for COX-2 was performed according to Pi et al. After deparaffinization and hydration, sections were incubated with 3% H_2O_2 for 5-10 min, followed by antigen retrieval in 0.01 M citric acid. After blocking in 5% BSA for 20 min, sections were treated with phospho-MAPK polyclonal antibody (1:1000) at 4°C overnight. Following incubation with HRP conjugated goat anti-rabbit IgG (1:1000) at 37°C for 20 min, visualization was performed with DAB, and counterstaining was performed with hematoxylin, followed by dehydration, transparentization and mounting. In blank control group, the primary antibody was replaced with

PBS. Positive cells had brown cytoplasm, intensity of Cox-2 immunoreactivity was measured and mounted using Canada balsam. Image analysis software (ImageJ 1.36b, http://rsbweb.nih.gov/ij) and expressed in percentage.

The immunohistochemical staining of for caspase-3 was performed according to (Bebars et al., 2017). 5 µm-thick sections were cut from the paraffin embedded blocks and stained by IHC technique according to data sheet (Cat. #RP-09605). The slides were incubated overnight at room temperature with a purified rabbit polyclonal antibody raised against caspase-3. It is received as 0.5 ml concentrated for use. The Envision method was used for detection of antibody binding. For negative control, the primary antibody was replaced by PBS. Finally, all sections were counter stained with Mayer's hematoxylin and examined by light microscopy. Positive cells had brown cytoplasm, intensity of caspase-3 immunoreactivity was measured and mounted using Canada balsam. Image analysis software (ImageJ 1.36b, http://rsbweb.nih.gov/ij) and expressed in percentage (Bressenot et al., 2009). Quantitative data were estimated in 5 different non-overlapped fields for the same slide of each animal, in each animal 5 slides were counted and a total number of 25 fields of each group were counted.

Statistical analysis

The collected data were statistically analyzed using SPSS program (Statistical Package for Social Science) version 18.0. Descriptive statistics were given as mean ± standard deviation (SD). One-way ANOVA were used to compare the mean values of more than two groups. Multiple comparisons were estimated by the least significant difference (LSD) test. A value of $p<0.05$ was accepted as statistically significant, a value of $p<0.001$ was accepted as highly statistically significant, and a value of $p>0.05$ was accepted as non-statistically significant. Spearman's rank correlation coefficient was used to analyze the association between serum hormonal and lipid levels against body mass index, and echocardiography (EF) against morphometric parameters.

RESULTS

Body and heart anthropometric measures analysis

Rats from the HFD group showed a highly significant increase in body weight compared with the rats from ND, CAP group. The mean of weight of the HFD group was 380±6 gm, while that of ND and CAP groups was 255±9 gm, 232±4 respectively. Consumption of capsaicin parallel with high fat diet in (HFD+CAP) group showed partial protection from weight gain, the average weight of the HFD+CAP group was 315±20 gm. In contrast to final body weight, the body length showed no significant difference between different study groups. Therefore, body mass index (BMI) showed a highly significant increase in the HFD group, the mean of BMI of the HFD group was 0.72±0.05 gm/cm^2, while that of ND, CAP, HFD+CAP groups were 0.48±0.03, 0.40±0.06 and 0.60±0.02 gm/cm^2 respectively (Table 1, Fig. 1 A, B, C).

Table 1. Anthropometric measures analysis in different study groups.

	Control group (n10)				
	ND subgroup (n10)	CAP subgroup (n10)	HFD group (n10)	HFD+CAP group (n10)	P value
Initial body weight (gm)	185±7	190±8	193±4	192±8	0.0653
Final body weight (BW) (gm)	255±9	232±4*	380±6*	315±20*, **	0.0000
Body length (BL) (cm)	23±3	24±5	23±4	23±3	0.9163
Body mass index (BMI) (gm/cm^2)	0.48±0.03	0.40±0.06*	0.72±0.05*	0.60±0.02*, **	0.0000
Heart weight (HW) (g)	1.2±0.04	1.3±0.06	2.6±0.16*	1.9±0.27*, **	0.0000
Heart wt./body wt. % (H/BW%)	0.53±0.03	0.56±0.04	0.68±0.22	0.6±0.14	0.0841

One-way ANOVA, and least significant difference (LSD) test, P > 0.05: no significant differences, P < 0.05: significant differences, P < 0.001: highly significant differences.
* significant vs ND subgroup; ** significant vs HFD group

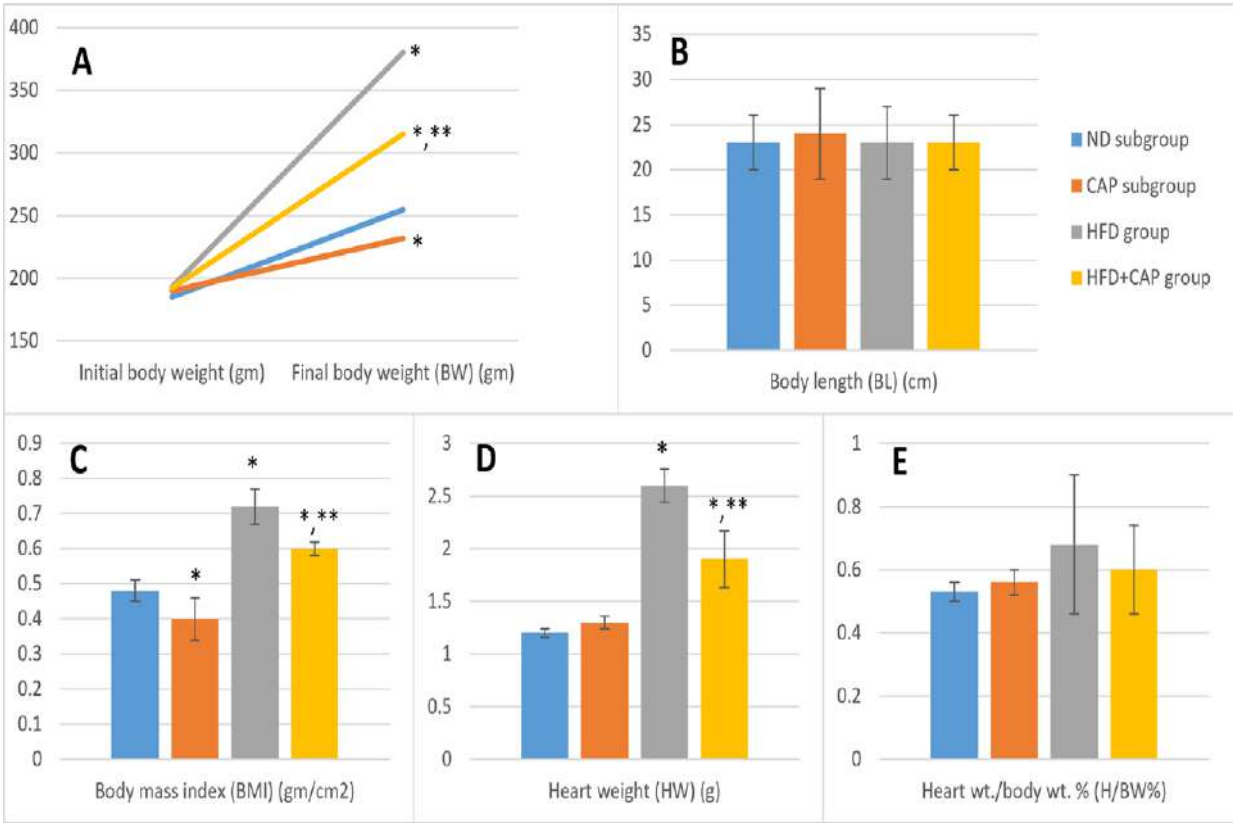

Fig. 1.- Anthropometric measures analysis in different study groups showed that rats from the HFD group showed a highly significant increase in body weight compared with the rats from ND, CAP group (**A**). In contrast to final body weight, the body length showed no significant difference between different study groups (**B**), therefore, body mass index (BMI) showed a highly significant increase in the HFD group (**C**). Moreover, Rats from the HFD group showed a highly significant increase in heart weight compared with the rats from ND, CAP group (**D**). In contrast to heart weight, the ratio between heart and body weights (H/BW%) showed no significant difference between different study groups (**E**).

Regarding heart measures, rats from the HFD group showed a highly significant increase in heart weight compared with the rats from ND, CAP group. The mean of heart weight of the HFD group was 2.6±0.16 gm, while that of ND and CAP groups was 1.2±0.04 gm, 1.3±0.06 respectively. Consumption of capsaicin led to partial protection, the average heart weight of the HFD+CAP group was 1.9±0.27 gm. In contrast to heart weight, the ratio between heart and body weights (H/BW%) showed no significant difference between different study groups (Table 1, Fig. 1 D, E).

Hemodynamic and Echocardiography parameters analysis

The rat fed with high fat diet exhibited significantly elevated heart rate, systolic blood pressure, LVESD, and significant reduction in EF% and FS% in comparison to both ND and CAP groups. These effects were alleviated by consumption of capsaicin parallel with high fat diet in (HFD+CAP) group (Table 2, Fig. 2).

Biochemical results analysis

Fasting serum glucose, insulin levels were significantly increased in HFD group as compared to ND and CAP groups. These effects were partially alleviated by consumption of capsaicin parallel with high fat diet in (HFD+CAP) group. With calculation of homeostasis model assessment of insulin resistance (HOMA-IR), the mean in rat on high fat diet was 3.8±0.4 while the mean in ND, CAP, HFD+CAP groups were 1.6±0.3, 1.5±0.3, 2.3±0.9 which indicate insulin resistance in HFD group (Table 3, Fig. 3 A, B, C).

The rat fed with high fat diet exhibited significantly elevated total cholesterol (TC), free fatty acids (FFA) and triglycerides (TG) in comparison to both ND and CAP groups. These effects were partially alleviated by consumption of

capsaicin parallel with high fat diet in (HFD+CAP) group (Table 3, Fig. 3 D, E, F).

Homogenate results analysis

Total energy charge was assessed by measurement of adenine nucleotide variants in the myocardia. The ATP and ADP concentrations in the myocardia of the HFD group were 2.23±0.36 and 2.17± 0.42 µg/ml, respectively, which were significantly lower than those of the other groups. On the contrary, the AMP concentration was significantly higher in the HFD group (1.54±0.27 µg/ml). Consequently, the HFD group had a significantly lower energy charge (0.57±0.15) vs 0.76±0.11, 0.81±0.15, 0.72±0.14 in ND, CAP,

Table 2. Hemodynamic and Echo parameters analysis in different study groups.

	Control group (n10)		HFD group (n10)	HFD+CAP group (n10)	P value
	ND subgroup (n10)	CAP subgroup (n10)			
Heart rate (beat/min.)	276±4	285±6**	312±6*	294±5*, **	0.0000
Systolic blood pressure (mmHG)	134±6	129±6**	181±4*	153±7*, **	0.0000
LVEDD	8.21±0.4	8.26±0.3	8.47±0.2	8.29±0.6	0.5079
LVESD	3.57±0.3	3.48±0.4**	4.04±0.5*	3.72±0.2	0.0090
EF%	71.5±3.4	68.3±2.6**	48.7±3.2*	68.5±4.2**	0.0000
FS %	36.84±2.1	35.46±3.7**	29.45±4.3*	32.65±3.5	0.0002

Left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD), ejection fraction (EF%), left ventricular percent fractional shortening (FS%).
One-way ANOVA, and least significant difference (LSD) test, P > 0.05: no significant differences, P < 0.05: significant differences, P < 0.001: highly significant differences.
* significant vs ND subgroup; ** significant vs HFD group

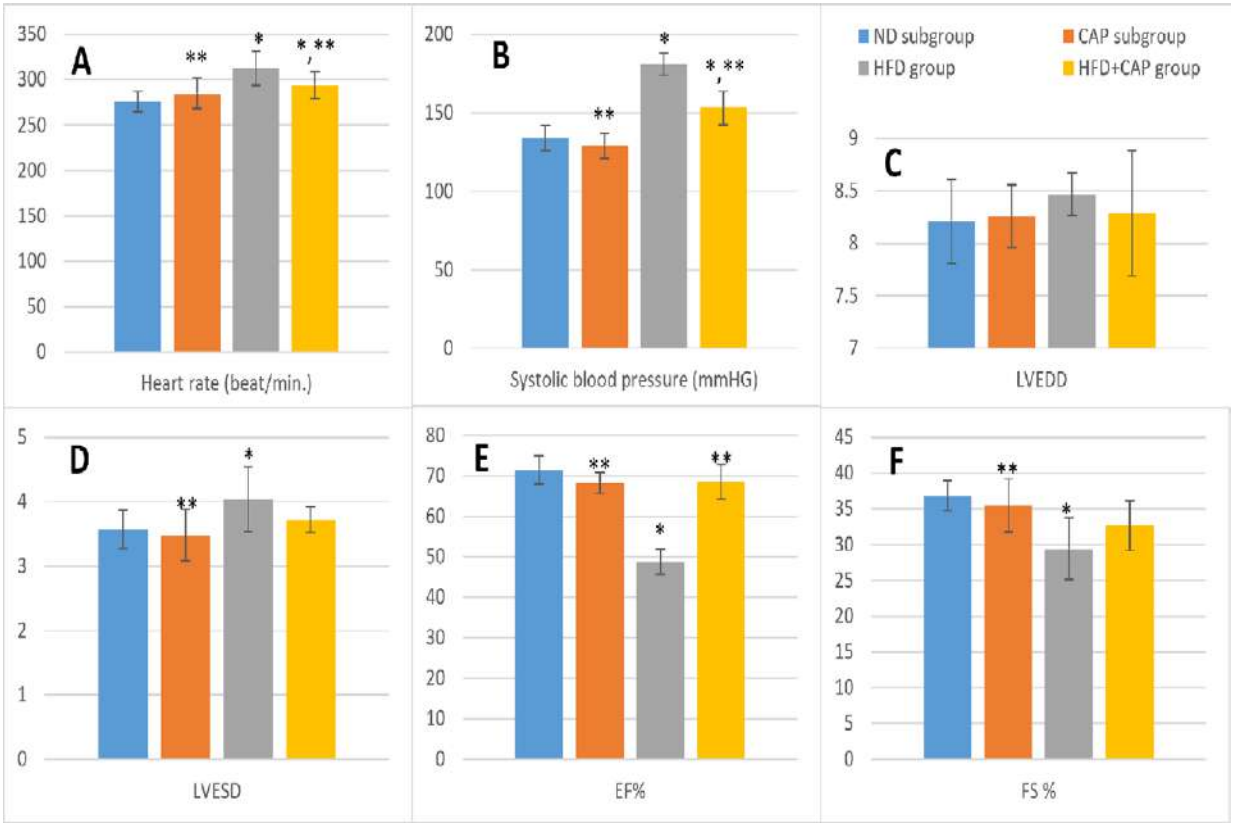

Fig. 2.- Hemodynamic and Echo parameters analysis in different study groups showed that rats fed high fat diet exhibited significantly elevated heart rate (**A**), systolic blood pressure (**B**), LVEDD (**C**), LVESD (**D**), and significantly reduction in EF% (**E**) and FS% (**F**) in comparison to both ND and CAP groups, these effects were alleviated by consumption of capsaicin parallel with high fat diet in (HFD+CAP) group.

HFD+CAP groups respectively (table 3, figure 3 G). Moreover, total antioxidant capacity was measured, the HFD group had an extremely significant lower antioxidant capacity (4.5±2.3) vs 15.7±2.2, 16.4±3.1, 12.4±2.7 in ND, CAP, HFD+CAP groups respectively (Table 3, Fig. 3H).

Table 3. Biochemical and homogenate results analysis in different study groups.

	Control group (n10)		HFD group (n10)	HFD+CAP group (n10)	P value
	ND subgroup (n10)	CAP subgroup (n10)			
Serum glucose level (mg/dl)	90±15	82±9**	150±23*	100±32**	0.0000
Serum insulin level (µU/ml)	7.2±0.8	7.6±1.2	10.4±0.5*	9.3±1.7	0.0000
HOMA-IR	1.6±0.3	1.5±0.3**	3.8±0.4*	2.3±0.9*, **	0.0000
Total cholesterol (TC) (mg/dl)	105±7	98±9**	182±12*	132±14*, **	0.0000
Free fatty acids (FFA) (µM/l)	0.34±0.11	0.29±0.08**	0.92±0.13*	0.45±0.23**	0.0000
Triglycerides (TG) (mg/dl)	64.6±2.14	54.5±1.75**	172±11.45*	112±13.45*, **	0.0000
ATP (µg/ml)	3.12 ± 0.37	2.98 ± 0.28	2.23 ± 0.36*	2.45 ± 0.36*, **	0.0000
ADP (µg/ml)	2.66 ± 0.22	2.56 ± 0.36	2.17 ± 0.42*	2.42 ± 0.15*, **	0.0000
AMP (µg/ml)	0.68 ± 0.21	0.62 ± 0.34	1.54 ± 0.27*	0.83 ± 0.23	0.0000
Total energy charge (µg/ml)	0.76 ± 0.11	0.81 ± 0.15**	0.57 ± 0.15*	0.72 ± 0.14	0.0030
Total Antioxidant Capacity (ng/mg)	15.7±2.2	16.4±3.1**	4.5±2.3*	12.4±2.7*, **	0.0000

One-way ANOVA, and least significant difference (LSD) test, P > 0.05: no significant differences, P < 0.05: significant differences, P < 0.001: highly significant differences.
* significant vs ND subgroup; ** significant vs HFD group

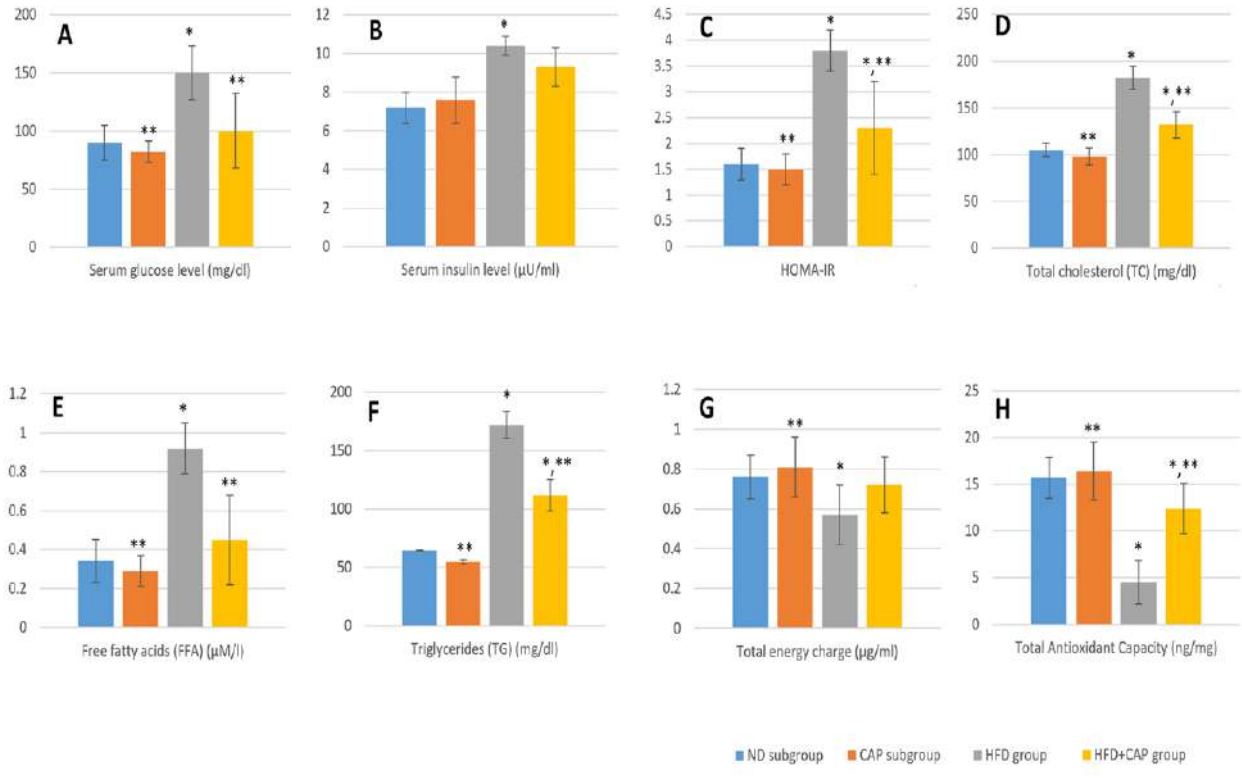

Fig. 3.- Biochemical and homogenate results analysis in different study groups showed that Fasting serum glucose (**A**), insulin levels (**B**) were significantly increased in HFD group as compared to ND and CAP groups. calculation of HOMA-IR (**C**) indicated insulin resistance in HFD group. Moreover, Rats from the HFD group exhibited significantly elevated total cholesterol (**D**), free fatty acids (**E**) and triglycerides (**F**). Total energy charge (**G**) and total antioxidant capacity (**H**) were significantly lower in HFD rats than those of the other groups.

Histological and morphometrical examination

Sections of the heart tissue in the ND group exhibited normal histological architecture of the myocardium as longitudinally striated, linearly arranged, branching and anastomosing muscle fibers (cardiac myofibers). They were joined together by intercalated discs and separated from each other by delicate layer of connective tissue. The cardiac muscle cells (cardiomyocytes) contained acidophilic cytoplasm with oval vesicular centrally located nuclei. (Fig. 4 A1, A2). In CAP group, the histological pattern of the heart tissue was similar to the ND group (Fig. 4 B1, B2). In the HFD group, alteration of the normal histological structure of the heart tissue was observed. Distorted cardiomyocytes with deeply stained pyknotic nuclei, vacuolated sarcoplasm, separation of the cardiac myofibers, inflammatory cellular infiltration, dilated congested blood vessels and extravasation of RBCs were observed (Fig. 4 C1, C2, E, F). In HFD+CAP group, improvement and restoration of the normal histological structure of the heart tissue was observed (Fig. 4 D1, D2). Myocyte cross-sectional area in the myocardia of the HFD group was 735±114 um^2, which were significantly more than those of ND, CAP, HFD+CAP groups (412±75, 398±123, 512±65 respectively) (Fig. 4G).

Masson's trichrome stained heart tissue sections showed normally distributed collagen fibers in between the cardiomyocytes were observed in ND and CAP groups (Fig. 5 A, B), but markedly increased bundles of collagen within the distorted cardiac tissue were observed in the HFD group (Fig. 5 C, D). Nearly normal appearance was observed in the HFD+CAP (Fig. 5E). Also, Area % collagen fiber was significantly increase in HFD group (25.7±8.3) in contrast to ND, CAP, HFD+CAP groups (16.5±3.4, 17.4±5.6, 21.2±7.4 respectively) (Fig. 5F).

Immunohistochemical staining reflected increase in the immunopositivity of the inflammatory marker COX-2 and the apoptotic marker caspase-3 in HFD group compared to other groups. These effects were partially alleviated by consumption of capsaicin parallel with high fat diet in (HFD+CAP) group, active cells appeared as brown stained in contrast to healthy cells that appeared blue with counter stain (Fig. 6).

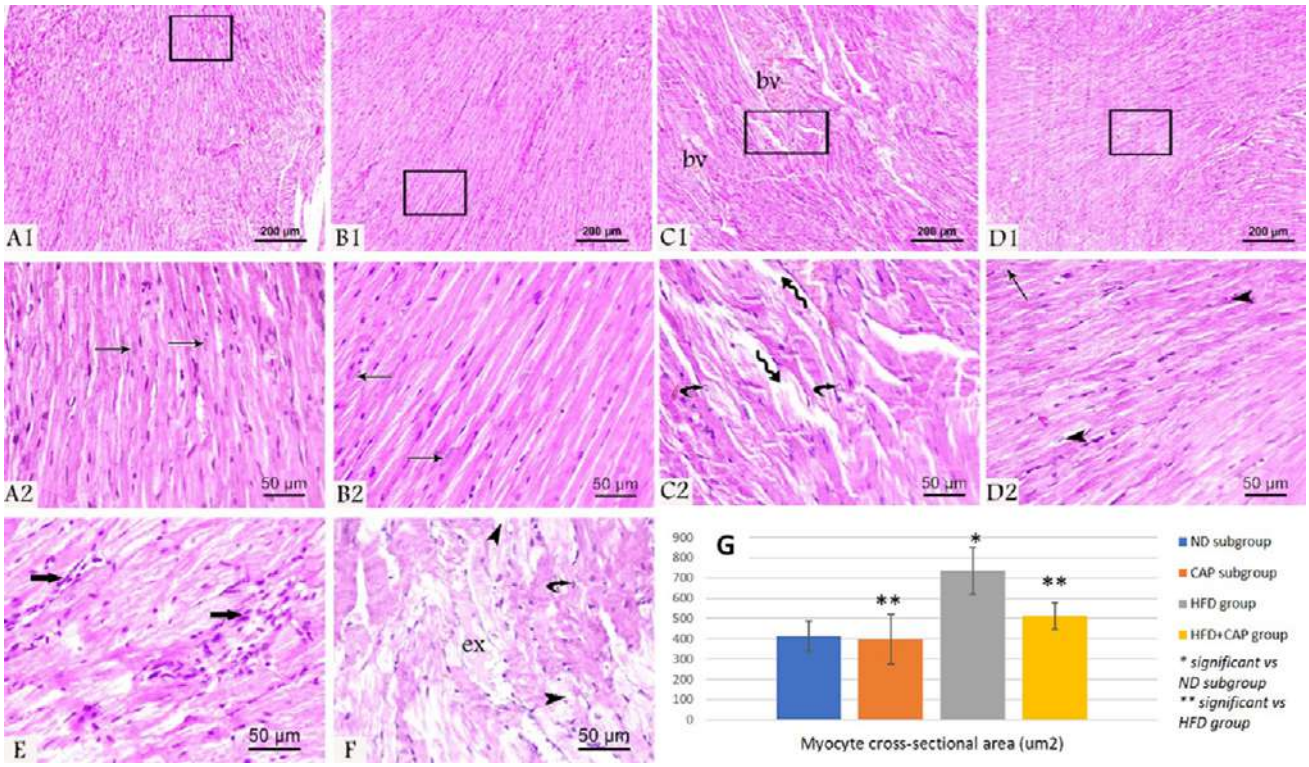

Fig. 4.- H&E-stained heart tissue sections from all the groups showing linearly arranged cardiac muscle fibers contained acidophilic cytoplasm with oval vesicular centrally located nuclei (arrow) in the ND (**A1, A2**) and CAP (**B1, B2**) groups. Notice the distorted cardiac muscle fibers, pyknotic nuclei (curved arrow), vacuolated sarcoplasm (arrowhead), inflammatory exudate (ex), dilated congested blood vessels (bv), inflammatory cellular infiltration (thick arrow) and separated cardiac muscle fibers (wavy arrow) in the HFD group (**C1, C2, E, F**). Restoration of the normal arrangement of the cardiac muscle fibers with oval vesicular centrally located nuclei (thin arrow) and some pyknotic nuclei (curved arrow) in the HFD + CAP group (**D1, D2**) are noticed. Morphometrical analysis showing increased myocyte cross sectional area in HFD group (**G**). Scale bars: A1, B1, C1 and D1 = 200 µm; A2, B2, C2, D2, E and F = 50 µm.

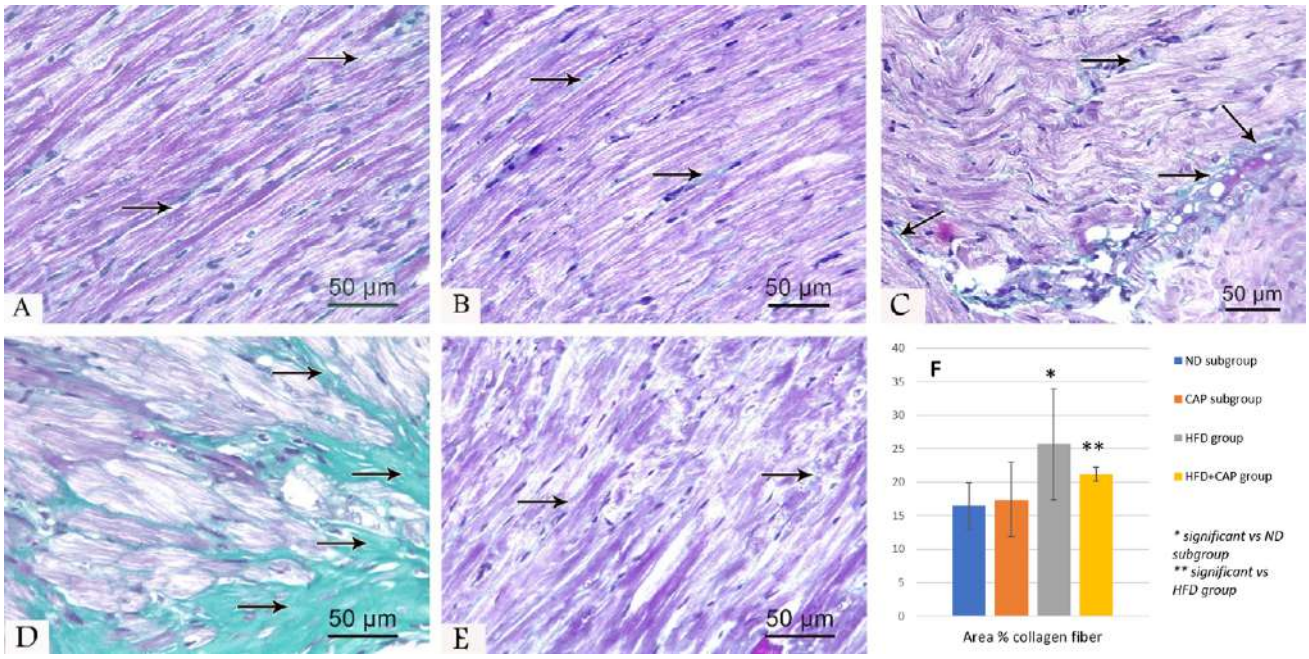

Fig. 5.- Masson's trichrome stained heart tissue sections from all the groups showing collagen fibers (arrow) which are normally distributed in the ND (**A**) and CAP (**B**) groups, markedly increased in the HFD group (**C, D**) and became normal in the HFD+CAP group (**E**). Morphometrical analysis showing significant increase of collagen fibers percentage in HFD group (**F**). Scale bars A-E = 50 µm.

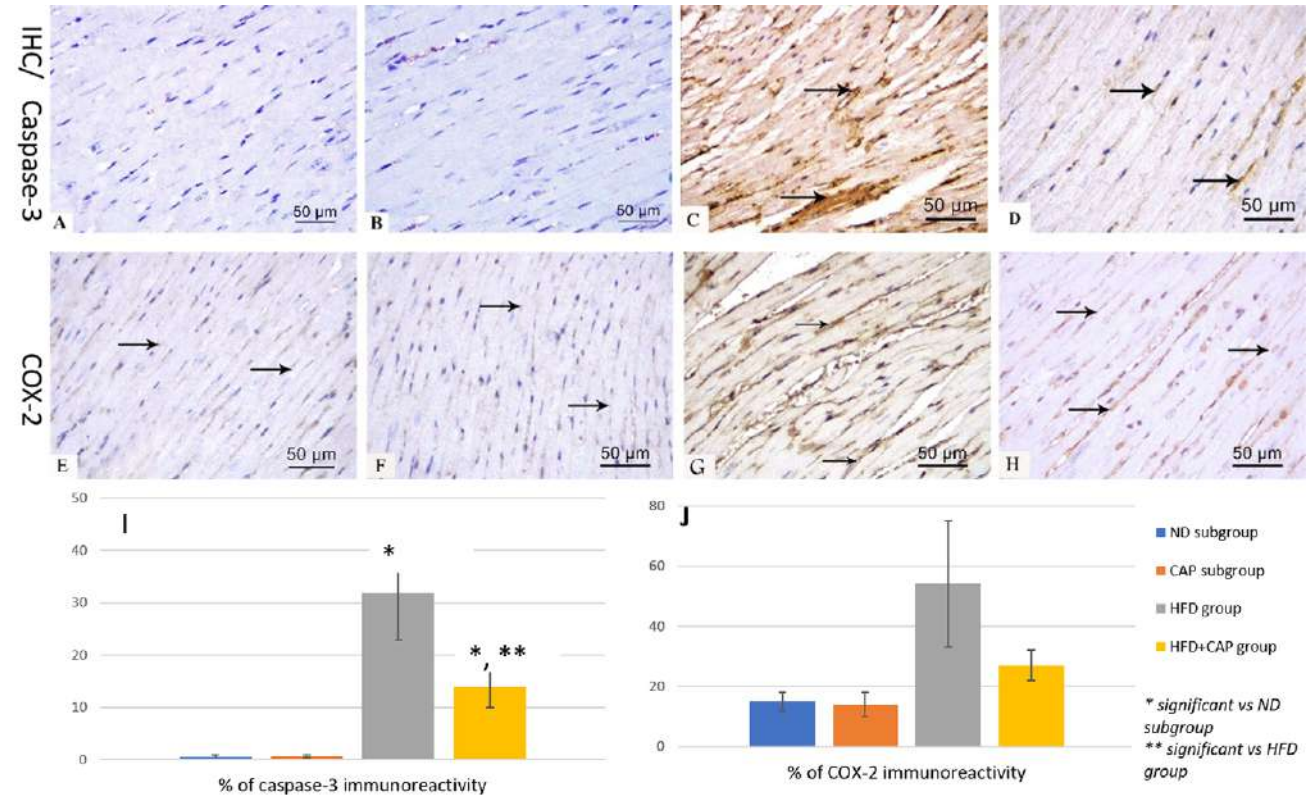

Fig. 6.- Caspase-3 (**A,B,C,D**) and COX2 (**E,F,G,H**) immuno-stained heart tissue sections from all the groups showing increased immunoreactivity (arrow) of both markers in HFD group (C,G) compared to other groups ND group (A,E), CAP group (B,F) and HFD+CAP group (D,H). Morphometrical analysis showing increased percentage of Caspase-3 (**I**) and COX2 (**J**) immunoreactivity in HFD group. Scale bars A-H = 50 µm.

Correlation between body mass index and other anthropometric and biochemical measures

Increase of body mass index in rats that were fed on HFD for 8 weeks was associated with significant positive correlation with heart weight (r=0.8303), heart wt. /body wt. % (r=0.5451), heart rate (r=0.8305) and systolic blood pressure (r=0.9406). Moreover, increase of body mass index in rats that were fed on HFD for 8 weeks was associated with significant positive correlation with serum biochemical parameters, serum glucose level (r=0.7568), serum glucose level (r=0.7568), serum insulin level (r=0.7093), total cholesterol (r=0.9066), free fatty acids (r=0.8268) and total triglyceride (r=0.9525) (Fig. 7).

Correlation between ejection fraction% and BMI and morphometric measures

Ejection fraction % as an indicator for left ventricular function showed a significant negative correlation with body mass index (r=-0.7697), heart rate (r=-0.8508), systolic blood pressure (r=-0.8721), myocyte cross sectional area (r=-0.8246), area % collagen fiber (r=-0.5271), % +ve COX2 cells (r=-0.7645) and % of apoptotic cells (r=0.7304). On the other hand, ejection fraction % showed a significant positive correlation with total energy charge (r=0.7016) and total antioxidant capacity (r=0.8938) (Fig. 8).

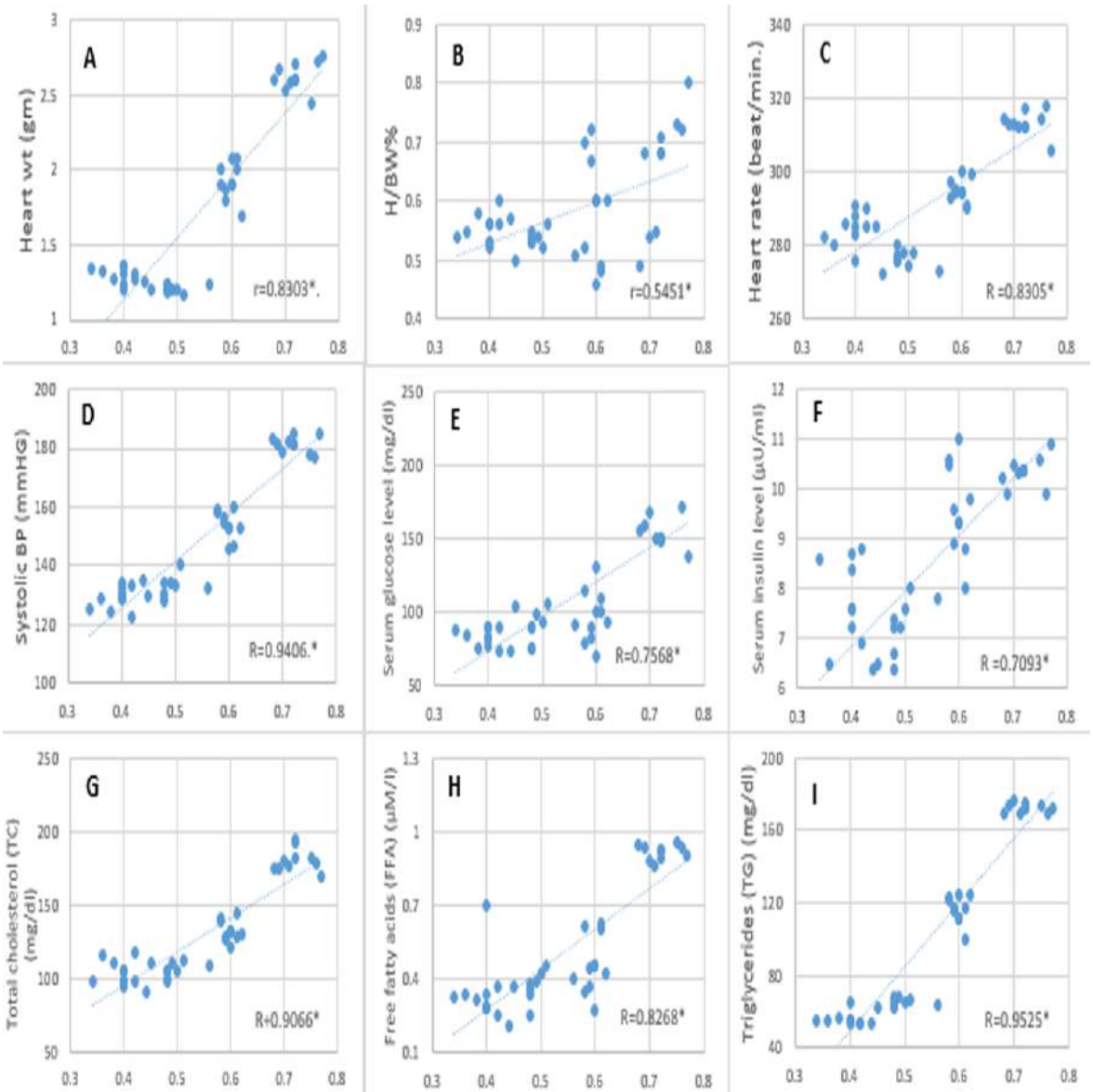

Fig. 7.- Correlation between body mass index and other anthropometric and biochemical measures, increase of body mass index in rats that were fed on HFD was associated with significant positive correlation with heart weight (**A**), heart wt. /body wt. % (**B**), heart rate (**C**) and systolic blood pressure (**D**). Moreover, increase of body mass index in rats that were fed on HFD was associated with significant positive correlation with serum biochemical parameters, serum glucose level (**E**), serum insulin level (**F**), total cholesterol (**G**), free fatty acids (**H**) and total triglyceride (**I**).

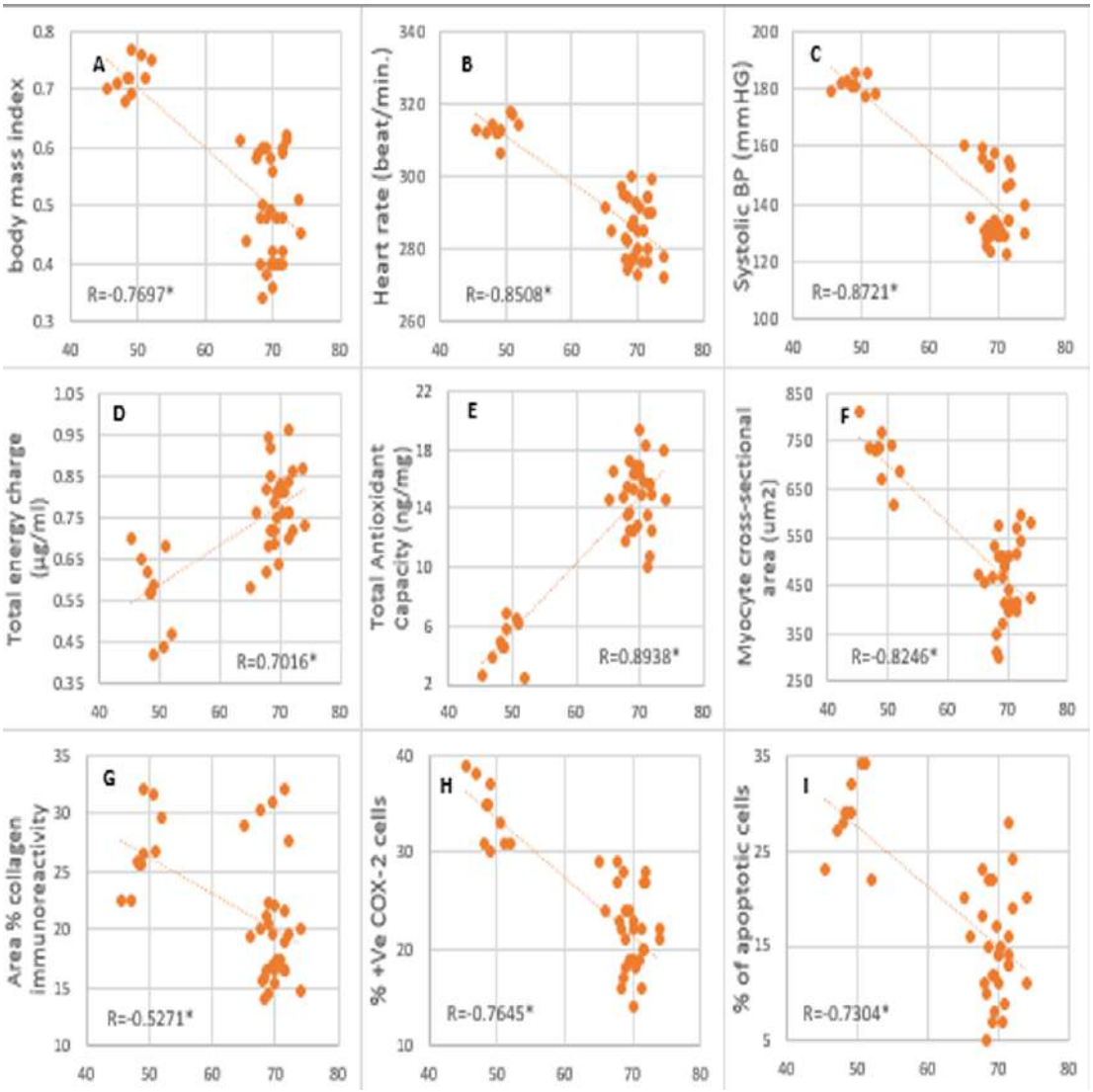

Fig. 8.- Correlation between ejection fraction% and BMI and morphometric measures showed a significant negative correlation with body mass index (**A**), heart rate (**B**), systolic blood pressure (**C**), myocyte cross sectional area (**F**), area % collagen fiber (**G**), % +ve COX2 cells (H) and % of apoptotic cells (**I**). On other hand Ejection fraction % showed a significant positive correlation with total energy charge (**D**) and total antioxidant capacity (**E**).

DISCUSSION

Obesity (Ob) has become one of the most prevalent metabolic diseases all over the world (Zhang et al., 2019). Ob is multifactorial in origin; overconsumption of high fat diet is a leading cause to develop obesity. Excess dietary fat increases both adipose and nonadipose tissue lipid content and, through lipotoxicity, leads to cell dysfunction and death, with or without the development of obesity (Hohos and Skaznik-Wikiel, 2017). Cardiovascular disorders are the critical causes of morbidity and mortality in association with Ob (Zhang et al., 2019). With worldwide overconsumption of high fat diet, it is mandatory to search about food additive which can make the balance and prevent cardiac dysfunction effect of HFD. So, it was aimed to achieve a new vision to the cardiac dysfunction induced by the effect of high fat diet through investigation of the hemodynamic, histopathological and biochemical changes in the heart of adult male rats, and to evaluate the potential ameliorative effect of capsaicin against this toxicity.

High fat diet used in the present study was sufficient to develop obesity in rats, Obese rats had an increase in body weight and heart weight, all these anthropometric parameters correlated directly with BMI, these results agreed with several studies (Carroll et al., 2006; Relling et al., 2006; Novelli et al., 2007; Bhandarkar et al., 2019; Mabrouki and Rjeibi, 2020) that induced obesity in rats through a hypercaloric diet. Further

hemodynamic analysis displayed significant increase in systolic heart rate and blood pressure as demonstrated by Wilde et al. (2000), Bhandari et al (2011), Bhandarkar et al. (2019), and Mabrouki and Rjeibi (2020): they reported significant increase in systolic, diastolic, mean arterial blood pressures and heart rate. Additional echocardiography examination to assess heart function in HFD rats revealed significant elevation of left ventricular end systolic diameter (LVESD), and significant reduction in left ventricular ejection fraction (EF%) and endocardial fractional shortening (FS%) in comparison to both ND and CAP groups. These results matched with Ge et al., (2012). Moreover, Li et al. (2018) observed enlarged LVESD, reduced EF and FS, but, in contrast with ours, this study, Li et al., observed also elevation of left ventricular end diastolic diameter (LVEDD).

Effect of HFD investigated biochemically and the results showed an elevation in fasting serum glucose, insulin levels in HFD group as compared to control groups. Calculation of homeostasis model assessment of insulin resistance (HOMA-IR), the mean in rat on high fat diet was 3.8±0.4, which indicates insulin resistance in HFD group. Similar biochemical results were reported by Mollica et al. (2017), Bhandarkar et al. (2019), Wang et al. (2021). Moreover, the rat fed with high fat diet exhibited significantly elevated total cholesterol, free fatty acids and triglycerides. All those biochemical parameters were in direct relation with body mass index, and this was corroborated by previous studied (De Leo et al., 2020; Mabrouki and Rjeibi, 2020; Wang et al., 2021).

Homogenate tissue analysis was done trying to understand the way by which HFD and obesity affect cardiac function, investigation of total energy charge showed that ATP and ADP concentrations in the myocardia of the HFD group were significantly lower than those of the other groups. On the contrary, the AMP concentration was significantly higher in the HFD group. Consequently, the HFD group had a significantly lower energy charge. These results suggested impaired mitochondrial dynamics, this was in accordance with Chen et al. (2018), who approved the effect of HFD in lowering total energy charge and impairment of mitochondrial dynamics.

This cardiac dysfunction has been described by many theories such as inflammation, LV fibrosis, collagen content alterations and increased oxidative stress (Sowers et al., 2011; Mollica et al., 2017). Many studies showed that oxidative stress and myocardial fibrosis are related to impairment of cardiac systolic function and insulin resistance (Whaley-Connell et al., 2007; Zhou et al., 2010; Begriche et al., 2013). So, our study investigated the total antioxidant capacity and the HFD group had an extremely significant lower antioxidant capacity, which is in accordance with (De Leo et al., 2020; Mabrouki and Rjeibi, 2020).

Further histological and morphometric examination of cardiac tissue observed that in HFD rats there were alteration of the normal histological structure of the heart tissue in form of increase myocyte cross-sectional area, distorted cardiomyocytes with deeply stained pyknotic nuclei, vacuolated sarcoplasm, separation of the cardiac myofibers, inflammatory cellular infiltration, dilated congested blood vessels and extravasation of RBCs, while Masson's trichrome stained heart tissue sections showed markedly increased bundles of collagen within the distorted cardiac tissue. Those histological results agreed with Sikder et al., (2018) and Bhandarkar et al., (2019), who interpreted the occurrence of cardiac fibrosis and the affection of cardiac performance by the excess deposition of collagen.

Immunohistochemical stained heart tissue sections in HFD rats reflected increase in the immunopositivity of the inflammatory marker COX-2 and the apoptotic marker caspase-3 in HFD group compared to other groups (Cole et al., 2011; Jørgensen et al., 2017), mentioned that COX2 was elevated with HFD with mitochondrial impairment and oxidative stress. Because inflammation is often associated by apoptotic events, the immunopositivity of caspase-3 was markedly increased in HFD group and this was in accordance with Fang et al. (2008) and Brugman (2016). Also settled with Li et al. (2018), who reported excessive apoptosis, deposition of collagen fibers and lipid droplets in cardiomyocytes.

Correlation between ejection fraction % as an indicator for left ventricular function and other findings showed a significant negative correlation with body mass index, heart rate, systolic blood pressure, myocyte cross sectional area, area % collagen fiber, % +ve COX2 cells and % of apoptotic cells. On the other hand, ejection fraction % showed a significant positive correlation with total energy charge and total antioxidant capacity. This was in agreement with Ge et al. (2012), who found negative affection of the cardiac function and reductions in EF and FS that were strongly negatively correlated with body weight and cardiac triglycerides content. They attributed these changes to impaired insulin resistance and increased oxidative stress that was also previously concluded by Manrique et al. (2013),; Pakdeechote et al. (2014), whose results convene with ours.

Cardiac dysfunction effects were partially alleviated by consumption of capsaicin parallel with high fat diet. Consequently, consumption of spicy foods as a food additive can make moderate balance and moderately prevent the harmful effect of HFD on heart. This conclusion is compatible with Almaghrabi et al., (2014), Sun et al. (2016) and Zheng et al. (2017), who conveyed that Capsaicin improves insulin resistance, decreases adipogenesis, regulates glucose homeostasis and ameliorates vascular dysfunction. Capsaicin guards against cardiometabolic diseases, such as obesity, hypertension, dyslipidemia in different target organs or tissues. Moreover Baskaran et al. (2019) suggested that CAP antagonizes HFD-induced metabolic stress and inflammation. Also, Kursunluoglu et al. (2018) investigated results in co-administration of capsaicin with cisplatin, and suggested that the antioxidant capacity of capsaicin alleviates the cardiotoxic effect of cisplatin. Gómez-Sierra et al. (2018) argue that capsaicin has analgesic, antimicrobial, anti-inflammatory, and antioxidant properties.

In summary, the high fat diet group showed significant increase in body weight, heart weight and body mass index. With hemodynamic and echocardiography investigation, HFD group showed significant increase in heart rate, systolic blood pressure with decrease in EF%, serum biochemical investigation revealed significant increase in serum glucose, insulin level with insulin resistance as well as increase in serum total cholesterol, triglyceride, free fatty acids. HFD heart homogenate showed decrease in total energy charge and total antioxidant capacity. Moreover, histological examination of heart tissue showed alteration of the normal histological structure of the heart tissue, marked increase of collagen bundles in the distorted cardiac tissue and significant increase in the immunopositivity of the inflammatory marker COX-2 and the apoptotic marker caspase-3 in HFD group compared to other groups. These effects were partially alleviated by consumption of capsaicin parallel with high fat diet. In brief, even though capsaicin could ameliorate effect of high fat diet, it is better to avoid HFD as it can induce cardiac dysfunction.

CONCLUSION

Even though capsaicin could ameliorate effect of high fat diet, it is better to avoid HFD as it can induce cardiac dysfunction.

AUTHOR CONTRIBUTIONS

All authors have been personally, equally, and actively involved in substantive work leading to the manuscript and will hold themselves cooperatively and personally accountable for its content.

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