



## Ameliorative effect of celery seed oil against high-fat diet-induced lipotoxic heart disease in rats

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### ABSTRACT

Obesity is a risk factor for heart disease. Persistent exposure to excess lipids in the circulation can harm the heart by accumulating hazardous metabolic by-products such as reactive oxygen species and ceramides, which can cause myocyte malfunction and death by activating signaling pathways. Herbs are one of the most popular weight-loss alternative medicines in the world. Our study aimed to evaluate the possible effects of celery seed oil against high-fat diet-induced lipotoxicity of heart in rats. The study was conducted on 40 male Wistar rats that were classified into 2 groups. Group I (GpI) served as control. GpII was obese rats, subdivided into four subgroups (untreated, treated with orlistat drug, celery seed oil, and a combination of celery seed oil /orlistat drug. At the end of treatment, all rats were scarified, blood samples and heart tissues of the rats were collected. Celery seed oil showed ameliorative effect against high-fat diet-induced lipotoxic heart disease through multiple interrelated mechanisms including alleviation the histological changes, boosting the antioxidant status, improving lipid profile, and reducing fatty acid accumulation in cardiac tissue. The treatment with a combination of celery seed oil /orlistat drug showed the best effect. In conclusion, celery seed oil could be used for the protection against obesity alone or used as adjuvant with orlistat drugs to enhance their pharmacological effect against lipotoxic heart disease.

**Keywords:** Celery seed oil, Lipotoxic heart disease, Obesity, Orlistat.

## 1. Introduction

Obesity is a double burden of malnutrition; its prevalence has risen in both sexes and all ages, regardless of race, geographic location, or socio-economic level. Obesity prevalence climbed from 0.8% in 1980 to 4.9% in 2015. Obesity or overweight affects more than a third of the world's population. If current trends continue, researchers predicted that the prevalence of adult obesity would approach 50% by 2030 (Boutari and Mantzoros, 2022). Egypt is the 14<sup>th</sup> fattest country in the world, according to (WHO) statistics among Egyptians whose age above 20, females are more overweight and obese than males. WHO

statistics estimated that 76.9 % of females in this age are overweight and obese, in comparison with over 62.4% of males. In 2016, approximately 340 million adolescents and children aged 5 to 19 were fat or overweight, including 41 million children under the age of five (Aboulghate et al., 2021). Obesity causes early death, according to the global burden of illness, with approximately 4 million people dying each year because of being fat or overweight (Ritchie and Roser, 2017). Obesity is a major risk factor for heart disease. Several studies in humans, nonhuman primates, and rodents showed a link between maternal obesity and offspring's risk of cardiovascular disease (Ferey et al., 2019). Persistent exposure to

excess lipids in the circulation can harm the heart by accumulating hazardous metabolic by-products such as reactive oxygen species (ROS) and ceramides, which can cause myocyte malfunction and death by activating signaling pathways (D'Oria et al., 2020). Obesity causes hematological, biochemical, and hepato-renal dysfunctions (El-Naggar et al., 2021).

Nonadipocytes have a very limited capacity to store excess fat. If they are exposed to high levels of plasma lipids, as usually occurs in obesity, they may undergo steatosis and loss of function, and ultimately fatty acid (FA)-induced 'lipoapoptosis', then triacylglycerol (TAG) accumulates rapidly in the heart, as it does in other non-adipose tissues, as the animals become increasingly obese (Poirier and Després, 2003). This is the consequence not only of elevated plasma lipids, but also of increased expression in non-adipose tissues of lipogenic enzymes such as glycerol-3-phosphate acyltransferase (GPAT), an enzyme of FA esterification, coupled with decreased expression of acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase-1 (CPT-1) enzymes of FA oxidation and their transcription factor, peroxisome proliferator-activating receptor- $\alpha$  (PPAR $\alpha$ ) (Marín-Royo et al., 2018). These changes are associated with a progressive increase in ceramide content and inducible nitric oxide synthase (iNOS) expression. A causal link between steatosis and cardiac dysfunction. TGs themselves are probably inert, but their hydrolysis to fatty acyl-CoA provides increased substrate for ceramide synthesis. Ceramide up-regulates iNOS expression and increases nitric oxide (NO) production; the resulting increase in peroxynitrite is believed to cause apoptosis. So, the increase in myocardial ceramide content is likely to reflect de novo synthesis. The extent of the myocardial apoptosis and the accompanying secondary fibrosis observed in this study certainly could account for a loss of cardiac function (Zhou et al., 2000). In general, people employ a variety of strategies to avoid the adverse effects and/or failure of standard weight-loss medications, such as meal replacements, exercise, and herbs. Herbs are one of the most popular weight-loss alternative medicines in the world. The bulk of these herbs

are utilized as raw plants, and herbs have been used to cure obesity all over the world in recent years. Efficacy and safety of medicinal plants in the treatment of obesity, dyslipidemia, and diabetes mellitus have been studied extensively (Genatrika et al., 2019).

One of the annual or perennial plants that grows throughout Europe as well as the tropical and subtropical parts of Africa and Asia is celery (*Apium graveolens* L), a member of the Apiaceae family. This plant's essential oils, seeds, and leaves are the portions that are used. Celery contains a variety of phytochemical substances, including alkaloids, steroids, carbohydrates, and phenols like flavonoids. Some main constituents of celery with the chemical structures can prevent cardiovascular diseases, jaundice, liver and lien diseases, urinary tract obstruction, gout, and rheumatic disorders (Feng et al., 2018). Our study aimed to evaluate the possible effects of celery seed oil against high-fat diet-induced lipotoxicity of heart in rats.

## 2. Materials and Methods

### Animals

The study was conducted on 40 male Wister rats, 2 months old (150-250 g), purchased from the animal house of Medical Research Institute, Alexandria, Egypt. All rats had free access to food and water with 12:12 hours light/dark cycle and constant environmental conditions prior to experimentation and thereafter. The current protocol was approved by Alexandria University-Institutional Animal Care and Use Committee (Alex-IACUC, Approval number: AU0122021112). All experiments fulfil the guidelines of the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) and the recommendations of Egypt's guide for the care and use of laboratory animals. All efforts were made to curb the distress of rats during the experimental period, by using best practices for commonly used procedures, such as blood sampling and feeding rats with a consistent supply of food and water, and simultaneously cleaning their cages, which can enormously improve animal welfare.

### Induction of obesity

Obesity was induced in male Wister rats by feeding them with a high-fat diet for 3 months. Rats that were 20% heavier than control rats of the same age were considered obese (Kamel et al., 2014). High fat was prepared by mixing sheep ghee and coconut oil in the ratio of 3:1 (v/v) (Ahmedy, 2016).

### Treatment

A Celery seed oil was purchased from Sigma Aldrich and given to rats orally by gastric tube in a dose of 300 mg/kg body weight (b.wt./day) for one month (Ahmedy, 2016).

Orlistat (a product of October pharma) was available in the form of tablets. These tablets were dissolved in distilled water and given to rats orally by gastric tube in a dose of 30 mg/kg b.wt./day for one month (Gomaa et al., 2019).

### Experimental design

The rats were divided into two groups: GpI was served as control group, consists of 8 healthy male rats that were fed a normal diet. GpII (Obese group): Consists of 32 obese rats that were subdivided into four subgroups (8 rats each). GpIIA: Untreated obese rats. GpIIB: Obese rats that were orally treated with orlistat drug 30 mg/kg b. wt. /day for four weeks. GpIIC: Obese rats that were orally treated with celery seed oil in a dose of 300 mg/kg b. wt. /day for four weeks. GpIID: Obese rats that were orally treated with celery seed oil (300 mg/kg b. wt./day) and orlistat (30 mg/kg b. wt./day) for four weeks. At the end of treatment period, all rats were sacrificed under deep anesthesia using isoflurane with concentration of 100% to obtain blood and heart tissues were collected for biochemical analysis to detect the progression of obesity.

### Sample preparation

The blood samples were collected in tubes, left for 20 min at room temperature and centrifuged at 1000 xg for 10 minutes to obtain serum for assessment of fasting blood sugar, insulin, lipid profile, the homeostasis model assessment index for insulin resistance (HOMA-IR), malondialdehyde (MDA) level, ferric reducing antioxidant power (FRAP), and nitric oxide end products (NOx).

Apart from heart tissue was rinsed with phosphate buffer saline (PBS) and then homogenized in PBS (1:9). After homogenization, the homogenate was centrifuged at 1400 xg, at 4°C for 20 minutes. The supernatants were divided into aliquots and stored at -20°C for subsequent determination of ceramide, triglyceride (TG), free fatty acid (FFA), and NOx. Approximately, 30 mg of obtained tissues were used for RNA extraction and determination the gene expression of PPAR $\alpha$ , and iNOS.

### Sample preparation for histopathological observations

The heart tissue specimens were collected from different rat groups, rinsed in saline solution, and then immediately fixed in 10% buffered formalin (pH 7.4) for at least 24 h. The fixed tissue specimens were processed through the conventional paraffin embedding technique. The paraffin sections (4.5  $\mu$ m) were dewaxed and stained with Mayer's hematoxylin and eosin (H&E) stain. Stained sections were examined by light microscope and photographed using a digital camera (Nikon Corporation Co., Ltd., Japan).

### Serum parameters

Serum levels of fasting blood glucose (FBG), aspartate aminotransferase (AST) activity, TG, total cholesterol (TC), and high-density lipoprotein-cholesterol (HDL-C) were assayed using commercially available kits (Bio-Med Diagnostic INC, USA). Low-density lipoprotein-cholesterol (LDL-C) was estimated according to Friedewald's equation, LDL-C (mg/dl) = TC - (HDL-C) - (TG/5). All procedures were performed according to the manufacturer's instructions. HOMA-IR was then calculated using the following formula (Caumo et al., 2006).

$$HOMA - IR = \frac{Fasting\ insulin \left( \frac{\mu IU}{ml} \right) \times Fasting\ glucose \left( \frac{mg}{dl} \right)}{22.5 \times 18}$$

Determination of creatine kinase-MB (CK-MB) concentration was measured by a micro particle assay on an IMx (Abbot Diagnostics, Berkshire, UK) following the manufacturer's recommended method. Determination of Troponin I (cTnI) was by an enzyme-linked immunosorbent assay (ELISA) using the ES-

300 Immunoassay analyzer (Boehringer Mannheim, Sussex, UK).

#### **Determination of malondialdehyde (MDA)**

The MDA level was determined according to the method of Draper and Hadley (1990). The tissue samples are heated with thiobarbituric acid (TBA) at low pH. The resulting pink chromogen has a maximal absorbance at 532 nm. An aliquot of 0.1 mL of the sample was pipetted into a tube containing an equal volume of SDS solution. This was followed by the addition of 0.75 mL of acetic acid, 0.75 mL of TBA, and 0.3 mL of distilled water. The contents of the tubes were then mixed with a vortex. The tubes were incubated in a boiling water bath for 1 h and then cooled to room temperature. An aliquot of 0.5 mL of distilled water was added to each tube, followed by the addition of 2.5 mL n butanol. The contents of the tubes were vigorously mixed with a vortex and then rotated in a centrifuge at 4000 rpm for 10 min. The absorbance of the organic layer was read at 532 nm using a spectrophotometer against a blank containing phosphate buffer solution instead of the sample.

#### **Determination of nitric oxide end products**

The NO<sub>x</sub> concentration was determined by simple Griess reaction (Guevara et al., 1998). Because the nitric oxide (NO) has a short half-life (2-30 sec), it is preferable to determine nitrite, the stable product of NO which may be further oxidized to nitrate. So, the Griess reaction was supplemented with the reduction of nitrate to nitrite by Cadmium beads.

#### **Determination of total antioxidant capacity**

The FRAP value as a measure of “antioxidant Power” (Hidalgo and Almajano, 2014) The method described measures FRAP at low pH, when a ferric- tripyridyltriazine (FeIII-TPTZ) complex is reduced to ferrous (FeII) form, an intense blue color with an absorption maximum at 593 nm develops.

#### **Determination of triglyceride content**

Hepatic triglyceride contents were determined using a minor modification of the Folch method (Folch et al., 1957). The amount of 50 mg of liver tissue was homogenized in 5 mL of a chloroform/methanol (2:1) mixture. The

extract was centrifuged at 2500 ×g for 15 min, and the supernatant was collected and evaporated to dryness under nitrogen. The residue was subsequently reconstituted in a solution of isopropyl alcohol containing 10% triton X and centrifuged at 10,000 ×g for 10 min. The supernatant was used for the determination of triglycerides using commercially available kits (Bio-Med Diagnostic Inc., White City, Oregon, USA

#### **Determination of free fatty acid (FFA) in heart tissues**

Colorimetric method was used for sensitive enzyme-based method quantification of FFA in rat heart tissues. This assay is a BioVision's Free Fatty Acid Quantification Kit provides a convenient, sensitive enzyme-based method for detecting the long-chain free fatty acids in various biological samples, Fatty Acids are converted to their CoA derivatives, which are subsequently oxidized with the concomitant generation of color or fluorescence. C-8 (octanoate) and longer fatty acids can then be easily quantified by colorimetric (spectrophotometry at 570 nm).

#### **Determination of ceramide (Cer) in heart tissues.**

This rat Cer ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Cer. (Cat no.: MBS7255105). The total protein concentration was determined using Lowry's method (Lowry et al., 1951)

#### **Gene expression analysis**

Thirty mg of the heart was used for total RNA extraction using the miRNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions and the concentration and integrity of extracted RNA were checked using nanodrop. The reverse transcription of the extracted RNA was performed using reverse transcription (RT) was performed by TOPscript™ RT DryMIX kit (dT18/dN6 plus) (Enzynomics, Korea) according to the manufacturer instructions. The tissues expression of *PPAR-α* and *iNOS* were quantified in the cDNA by CFX Maestro™ Software (Bio-Rad, USA) using QuantiNova™ SYBR® Green PCR Kit (Qiagen, Germany). Quantitative PCR amplification conditions were adjusted as an initial denaturation at 95°C

for 10 minutes and then 45 cycles of PCR for amplification as follows: Denaturation at 95 °C for 20 s, annealing at 55 °C for 20 s and extension at 70 °C for 15 s. The housekeeping gene 18S rRNA was used as a reference gene for normalization (Gowayed, et al., 2020). The primers used for the determination of rat genes are presented in Table (1). The relative change in mRNA expression in samples was estimated using the  $2^{-\Delta\Delta Ct}$  method.

### Statistical analysis

The Data were analyses using SPSS software package version 18.0 (SPSS Chicago, IL, USA). The data were expressed as mean  $\pm$  SD. The Kolmogorov-Smirnov test was used to study the normal distribution of the studied parameters. A one-way analysis of variance (ANOVA) was made and followed by post hoc Tukey test to compare the mean values between and within treated groups compared to untreated and control groups. Differences were considered statistically significant at P-value < 0.05. Correlation studies were performed using Pearson's correlation coefficient.

## 3. Results

### Glucose homeostasis parameters

The results indicated that FBG, insulin and HOMA-IR levels were significantly higher in obese untreated group compared to control group, they were significantly lower in both orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, they were statistically significantly lower in rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Table 2).

### Lipid profile

The current study indicated that total cholesterol, triglyceride and LDL levels were significantly higher in obese untreated group than the control group and significantly lower in both orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, total cholesterol, triglyceride and LDL levels showed a statistically significant lower in rats treated with orlistat and celery seed oil compared to celery seed oil treated group. The results of HDL levels were significantly lower in obese untreated group than the control group. While HDL levels were significantly higher in the

orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, HDL level showed a statistically significant higher in rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Table 3).

### Heart function tests

The results indicated that AST activity, CK-MB and troponin-I levels were significantly higher in obese untreated group compared to control group, they were significantly lower in both orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, they statistically significantly lower in rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Table 4).

### Redox parameters

The results indicated that MDA level was significantly higher in the obese untreated group than the control group. While MDA level was significantly lower in the orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, MDA level showed a statistically significant lower in a group of rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Table 5). The results also showed that the plasma level of TAC by FRAP was significantly lower in obese untreated rats compared to control group. While the level of TAC was significantly higher in obese rats treated with either orlistat or celery seed oil as compared to untreated group. Also, TAC level showed a statistically significant higher in the combined treated group. (Celery seed oil and orlistat) compared to celery seed oil treated group (Table 5).

### Cardiac content of triglyceride and free fatty acids

The present results showed a two-fold increase in cardiac TG content in HFD-feeding rats compared to normal rats, but the triglyceride content was significantly lower in the orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, triglyceride content showed a statistically significant lower in a group of rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group. The current results showed a two-fold increase in

cardiac FFAs content in obese rats compared to normal rats. While our results showed FFAs content was significantly lower in the orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, FFAs

content was statistically significantly lower in rats treated with a combination of orlistat and celery seed oil compared to the celery seed oil treated group (Table 6).

**Table 1.** Primer's sequence

Gene Name	Accession number	Primers	
18Sr RNA	NR_046237.2	F	5'- GTAACCCGTTGAACCCCAT -3'
		R	5'- CAAGCTTATGACCCGCACTT -3'
PPAR-alpha	NM_013124.3	F	5'- TGAACAAGACGGGATGATG-3'
		R	5'- TCACCTGCTGCTGATTCTC-3'
iNOS	NM_012611.3	F	5'- CTGTGGTCACCTATCGCACC-3'
		R	5'-AGCCACATCCCGAGCCATGC--3'

**Table 2.** Statistical analysis of glucose homeostasis; FBG, Insulin levels and HOMA-IR in the different studied groups.

Groups	FBG (mg/dl)	Insulin ( $\mu$ IU/ml)	HOMA-IR
Control	88.00 $\pm$ 6.16	9.77 $\pm$ 0.35	2.12 $\pm$ 0.12
Obese	122.25 $\pm$ 9.3*	23.25 $\pm$ 1.54*	7.02 $\pm$ 0.73*
Orlistat treated	93.4 $\pm$ 10.6 <sup>a</sup>	8.80 $\pm$ 0.42 <sup>a</sup>	2.03 $\pm$ 0.28 <sup>a</sup>
Celery seed oil treated	116.63 $\pm$ 7.21*	12.16 $\pm$ 0.62 <sup>ab</sup>	3.50 $\pm$ 0.27 <sup>ab</sup>
Orlistat+ Celery seed oil treated	82.50 $\pm$ 5.09 <sup>abc</sup>	8.77 $\pm$ 0.75 <sup>ac</sup>	1.79 $\pm$ 0.22 <sup>ac</sup>

The data represented means  $\pm$  S.D. FBG: Fasting blood glucose; HOMA-IR: Homeostatic model assessment for insulin resistance \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.

**Table 3.** lipid profile parameters in the different studied groups.

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	80.63 $\pm$ 1.41	64.89 $\pm$ 4.9	61.63 $\pm$ 1.6	6.02 $\pm$ 1.48
Obese	98.75 $\pm$ 10.1*	109.29 $\pm$ 14.5*	41.38 $\pm$ 3.8*	35.52 $\pm$ 7.49*
Orlistat treated	79.24 $\pm$ 3.0 <sup>a</sup>	71.63 $\pm$ 8.9 <sup>a</sup>	48.93 $\pm$ 3.0 <sup>a</sup>	15.99 $\pm$ 4.92 <sup>a</sup>
Celery seed oil treated	82.95 $\pm$ 8.7 <sup>a</sup>	61.20 $\pm$ 14.1 <sup>a</sup>	53.39 $\pm$ 8.2 <sup>a</sup>	17.32 $\pm$ 4.86 <sup>a</sup>
Orlistat+ Celery seed oil treated	79.88 $\pm$ 10.8 <sup>a</sup>	54.75 $\pm$ 13.3 <sup>ab</sup>	62.13 $\pm$ 9.8 <sup>abc</sup>	6.80 $\pm$ 1.49 <sup>abc</sup>

The data represented means  $\pm$  S.D. TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein cholesterol; LDL: Low density lipoprotein cholesterol; \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.

**Table 4.** AST activity, CK-MB and troponin-I levels in the different studied groups.

Groups	AST (mg/dl)	CK-MB (ng/ml)	Troponin I (ng/ml)
Control	92.62±3.92	0.74±0.21	0.52±0.07
Obese	145.62±8.95*	2.63±0.55*	1.32±0.48*
Orlistat treated	123.87±6.64 <sup>a</sup>	1.14±0.15 <sup>a</sup>	1.21±0.08*
Celery seed oil treated	91.25±5.39 <sup>ab</sup>	0.75±0.07 <sup>a</sup>	1.03±0.11 <sup>*ab</sup>
Orlistat+ Celery seed oil treated	75.5±6.39 <sup>*abc</sup>	0.75±0.08 <sup>a</sup>	0.89±0.05 <sup>*a</sup>

The data represented means ± S.D. AST: Aspartate aminotransferase; CK-MB: creatine kinase-MB; \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.

**Table 5.** Redox parameters in the different studied groups.

Groups	MDA (nmol/mg protein)	TAC In serum (μmole/ml)	TAC In cardiac tissue (μmole/ml)
Control	2.18±0.09	214.12±6.17	193.24±8.16
Obese	2.86±0.26*	183.13±4.19*	179.02±11.83*
Orlistat treated	2.04 ±0.40 <sup>a</sup>	194.87±5.00	198.34±11.67 <sup>a</sup>
Celery seed oil treated	1.56±0.39 <sup>*ab</sup>	202.55±4.02 <sup>*ab</sup>	184.93±11.79
Orlistat+ Celery seed oil treated	1.12 ±0.27 <sup>*abc</sup>	210.71±9.37 <sup>abc</sup>	214.67±9.00 <sup>*abc</sup>

The data represented means ± S.D. MDA: Malondialdehyde; TAC: Total antioxidant capacity; \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.

**Table 6.** Cardiac tissue triglycerides and fatty acid contents in the different studied groups.

Groups	TG (mg/dl)	FFA (nmol/mg protein)
Control	37.80±7.46	0.52±0.07
Obese	57.33±5.81*	1.32±0.48*
Orlistat treated	39.40±3.18 <sup>a</sup>	1.21±0.08*
Celery seed oil treated	43.44±5.36 <sup>*a</sup>	1.03±0.11 <sup>*ab</sup>
Orlistat+ Celery seed oil treated	35.20±3.44 <sup>ac</sup>	0.89±0.05 <sup>*a</sup>

The data represented means ± S.D. TG: Triglyceride; FFA: Free fatty acids; \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.

### **Cardiac ceramide content**

The current result showed a two and half- fold increase in cardiac ceramide content in obese rats compared to normal rats. We found that ceramide content was significantly lower in the orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, ceramide content showed a statistically significant lower in rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Fig.1).

### **Cardiac nitric oxide content, iNOS expression**

The results showed a four-fold increase in cardiac inducible nitric oxide synthase (iNOS) expression and two-fold increase in serum and cardiac nitric oxide (NO) levels in obese rats compared to normal rats. While iNOS expression was significantly downregulated in orlistat treated group and celery seed oil treated group compared to obese untreated group. Also, iNOS expression showed a statistically significant down-regulation in rats treated with orlistat and celery seed oil compared to celery seed oil treated group. While NO level in serum and its content in cardiac tissue were significantly higher in obese untreated group than the control group. Our results also showed NO was significantly lower in orlistat treated group and celery seed oil treated group compared to obese untreated group and highly significant lower in rats of combined group which treated with orlistat and celery seed oil compared to celery seed oil treated group (Fig . 2 and 3).

### **Cardiac peroxisome proliferator-activating receptor- $\alpha$ expression**

The results of the present study showed a four-fold decrease in cardiac PPAR $\alpha$  expression in HFD-feeding rats compared to normal rats. While PPAR $\alpha$  expression was significantly up regulated in orlistat treated group and celery seed

oil treated group compared to obese untreated group. Also, PPAR $\alpha$  expression showed a statistically significant up-regulation in rats treated with a combination of orlistat and celery seed oil compared to celery seed oil treated group (Fig. 4).

### **Histopathological findings and lesion scoring**

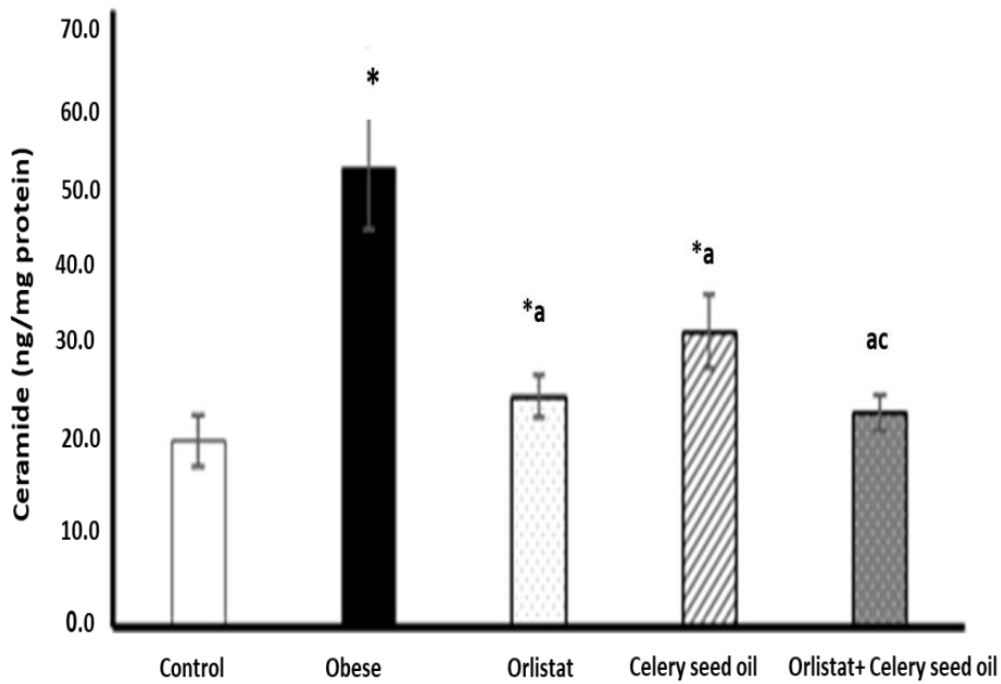
The histopathological examination of heart sections of the different experimental groups was shown in (Fig. 5 A-E). Heart of control group showing normal striated cardiac muscle fibers with their acidophilic sarcoplasm and centrally located nuclei (n) (Fig. 5A). Untreated obese group showing cardiac heart tissue with inter fibernum hemorrhage, separation of cardiac muscle fibers and discontinuation (Fig. 5B). Orally treated group with orlistat drug showing some congestion (Fig. 5C). orally treated group with celery seed oil for four weeks showing congestion and small separation (Fig. 5D). The combination group orally treated with celery seed oil and orlistat for four weeks showing cardiac muscle fibers with acidophilic sarcoplasm, centrally oval elongated nuclei and small area of hemorrhage (Fig. 5E).

### **Correlation studies**

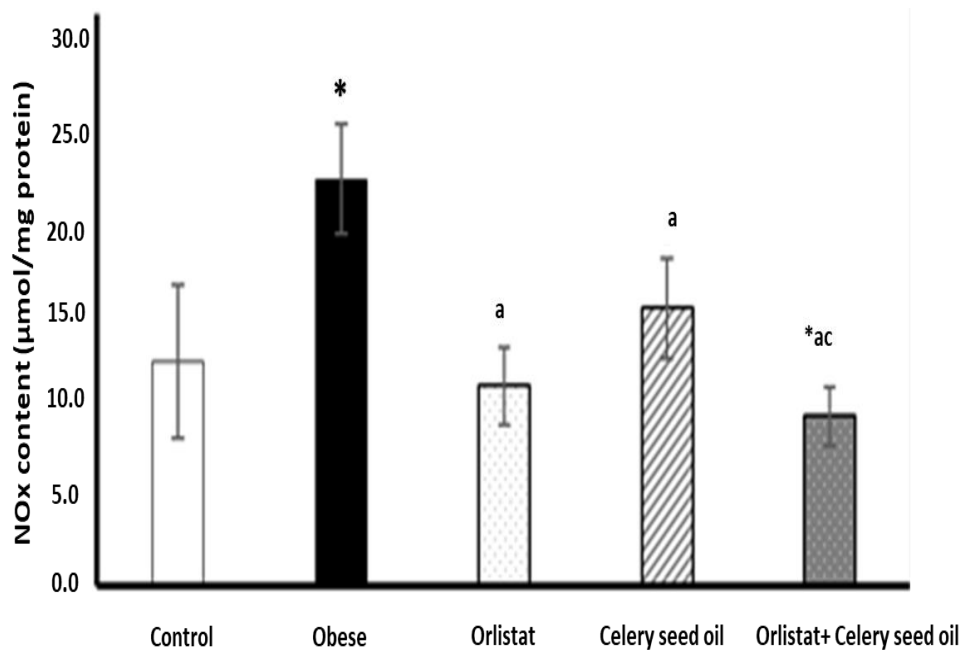
Statistical study by Pearson correlation showed the following:

Cardiac ceramide content was positively correlated with MDA level ( $r=0.65$ ,  $p=0.01$ , Fig. 6A), TG content ( $r=0.50$ ,  $p=0.01$ , Fig. 6B) and FFA content ( $r=0.80$ ,  $p<0.001$ , Fig. 6C) in celery seed oil treated groups. Cardiac iNOS expression was positively correlated with TG content ( $r=0.50$ ,  $p<0.001$ , Fig. 7A) and FFA content ( $r=0.50$ ,  $p<0.001$ , Fig. 7B) in celery seed oil treated groups. Cardiac PPAR $\alpha$  expression was negatively correlated with MDA content ( $r=0.50$ ,  $p<0.001$ , Fig. 8A), TG content ( $r=0.50$ ,  $p<0.001$ , Fig. 8B) and FFA content ( $r=0.50$ ,  $p<0.001$ , Fig. 8C) in celery seed oil treated groups.

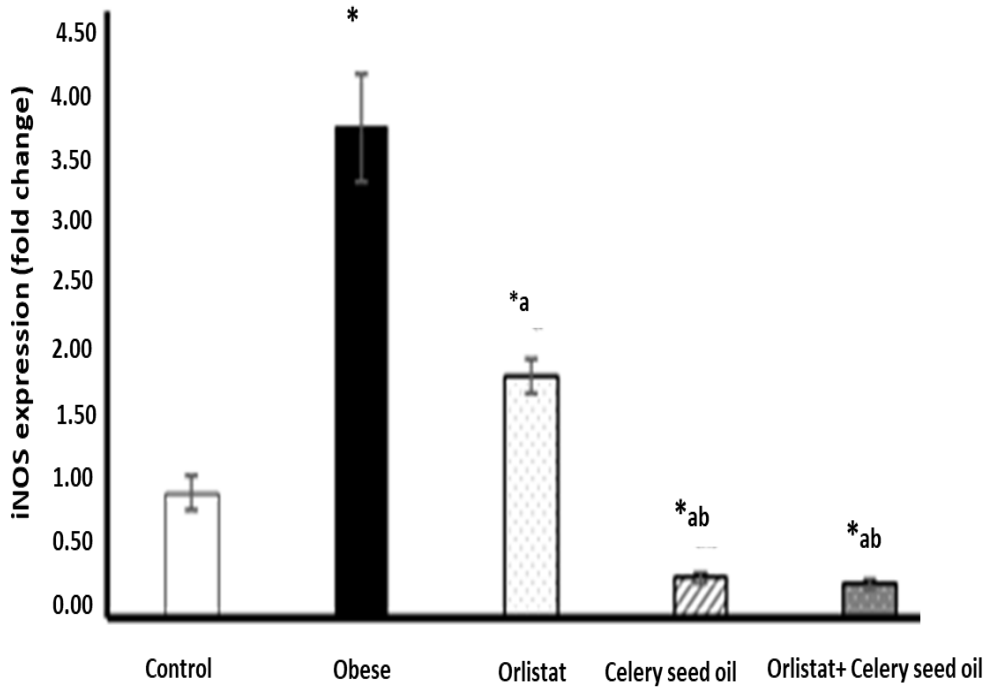




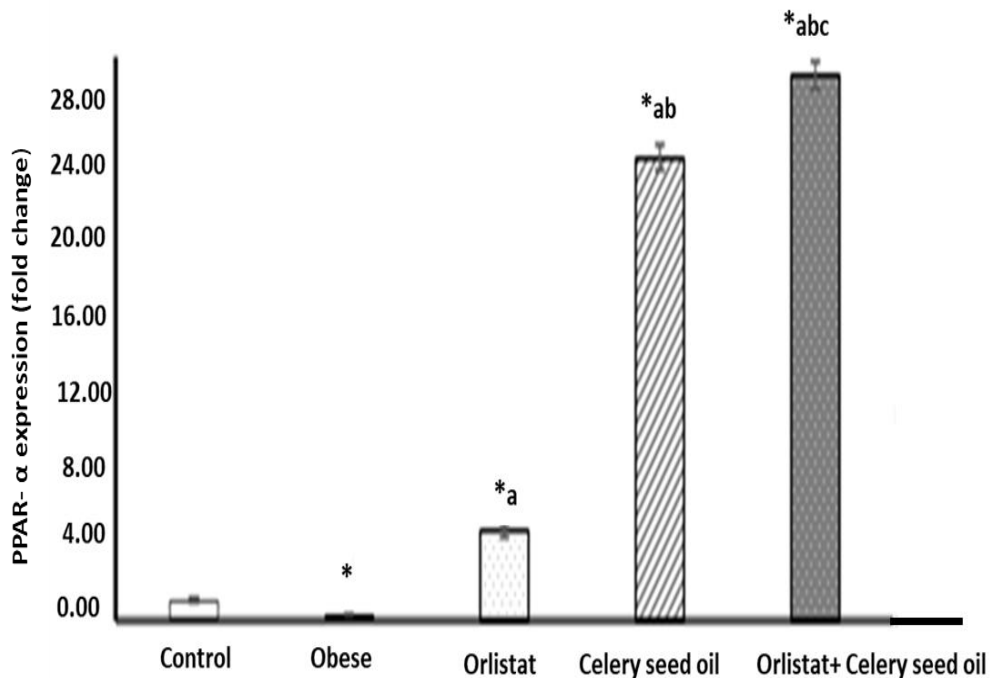
**Fig. 1:** Ceramide content in the different studied groups. \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.



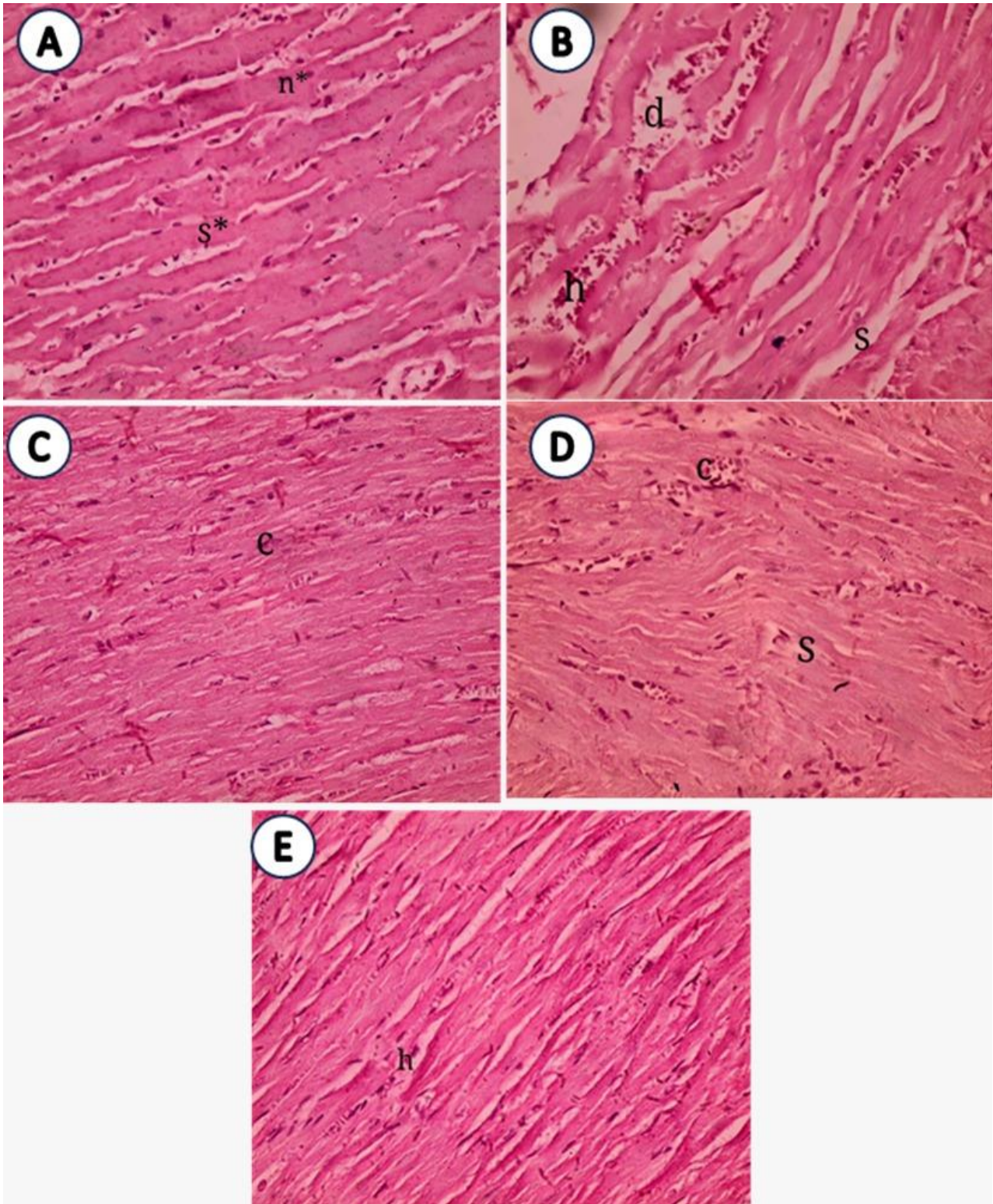
**Fig. 2:** Cardiac NOx content in the different studied groups. \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value < 0.05.



**Fig. 3:** Inducible nitric oxide synthase expression in the different studied groups. \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value< 0.05.



**Fig. 4:** Peroxisome proliferator-activating receptor-α expression in the different studied groups. \*: Significantly different from controls; a: Significantly different from untreated obese rats; b: Significantly different from orlistat treated rats; c: Significantly different from celery seed oil. The data was considered to be statistically significant at P-value< 0.05.



**Fig. 5:** Representative photomicrograph of rat hearts Control group (A), Untreated group (B), Orlistat treated group (C), Celery seed oil treated group (D), and Combination group (E). Striated cardiac muscle fibers with their acidophilic sarcoplasm (s) and centrally located nuclei (n), tissue with inter fibernum hemorrhage (h), separation of cardiac muscle fibers (s) and discontinuation (d) (HE,  $\times 400$ ).

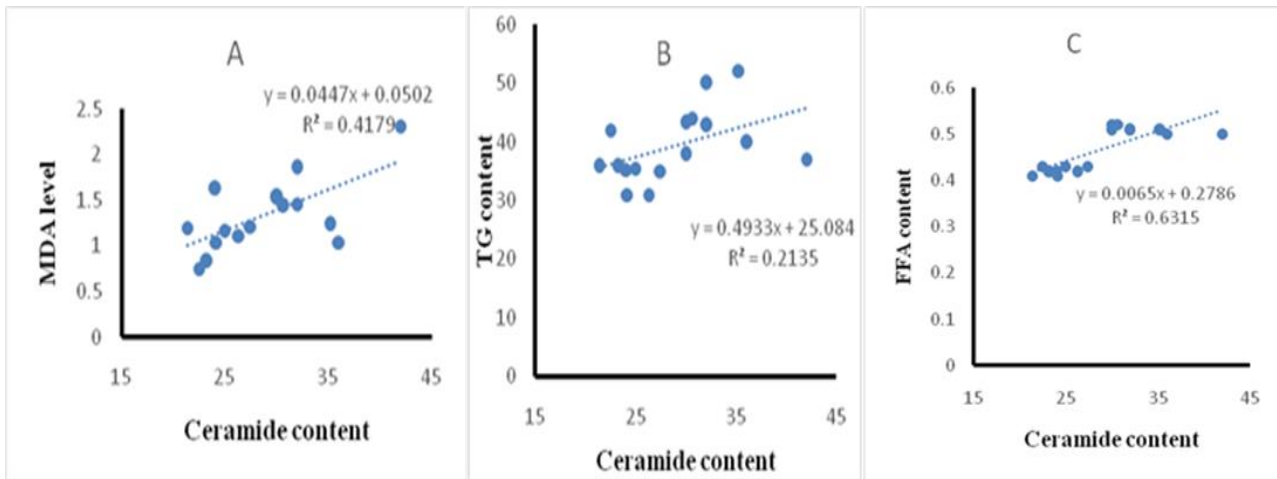


Fig. 6: Correlation study of cardiac ceramide content in celery seed oil treated groups

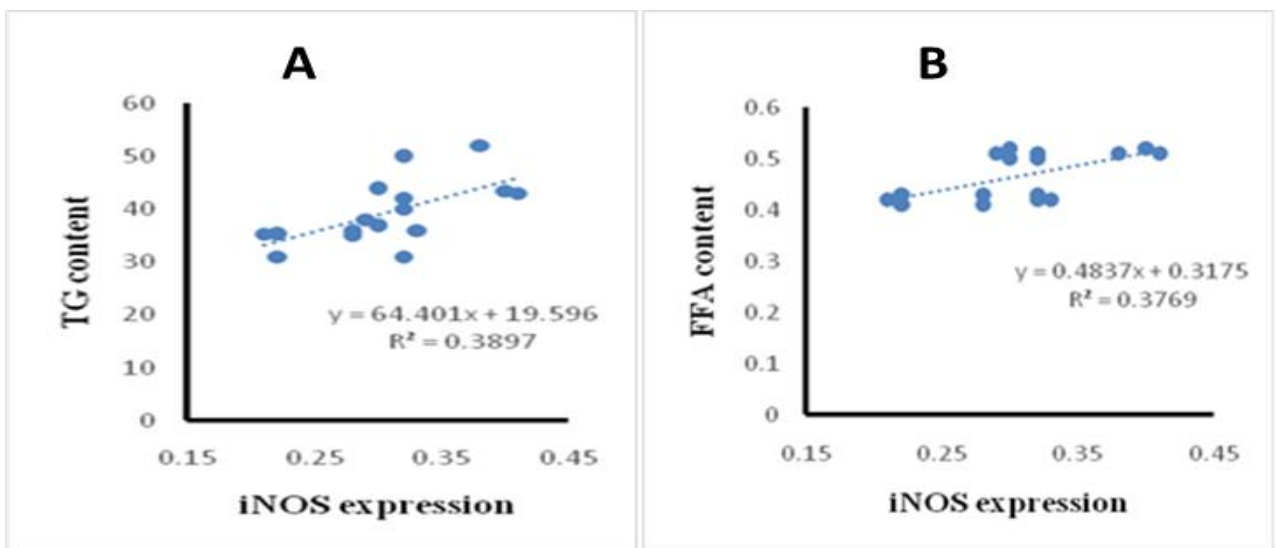


Fig. 7: Correlation study of cardiac iNOS expression in celery seed oil treated groups

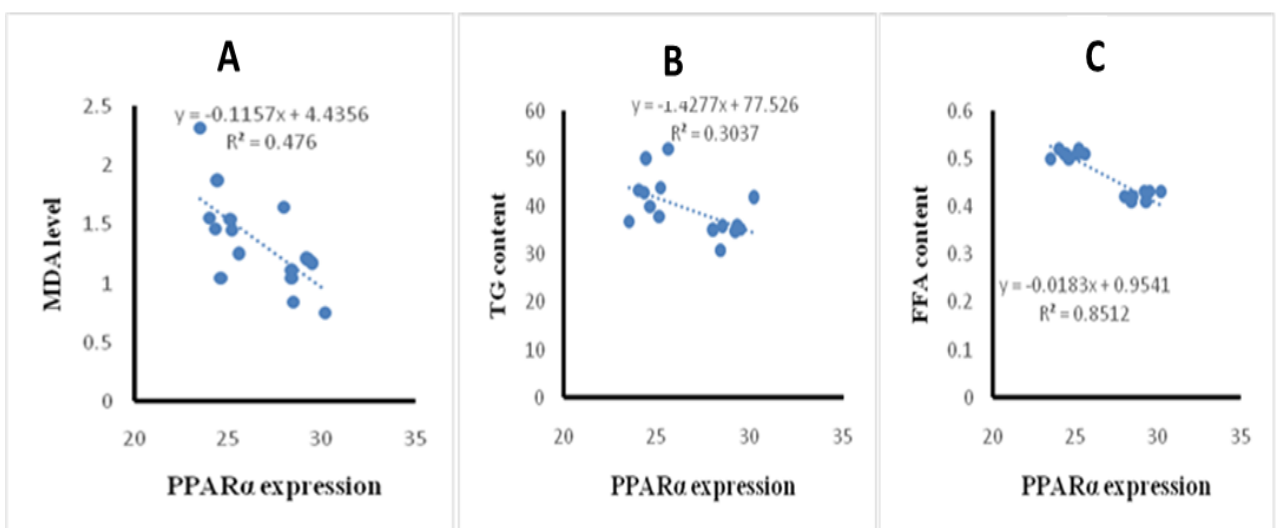


Fig. 8: Correlation study of cardiac PPARα expression in celery seed oil treated groups

#### 4. Discussion

In the present study, the HFD-obese rats developed the classical picture of obesity. The obese rats showed hyperglycemia and insulin resistance (elevated HOMA index) as the transformation from a metabolically stable condition to an obese and prediabetes state is characterized by a vicious loop that includes hyperinsulinemia, inflammation, glucose tolerance, dyslipidemia and insulin resistance (Petersen and Shulman, 2018).

Dyslipidemia is a well-known risk factor for cardiovascular disorders. It has been shown that, regardless of other risk factors, there is a substantial correlation between LDL and HDL cholesterol levels and CVD. This study's findings demonstrated that a high-fat diet significantly raised serum triglyceride and total cholesterol levels, which were further reflected in elevated LDL and decreased HDL values. These data suggested that a high-fat diet may cause systemic hypercholesterolemia that was prone to increased cardiovascular risk. Moreover, the high-fat diet-induced elevation of cardiac cholesterol levels at 4 weeks indicates that high-fat diet may directly lead to myocardial damage via excess cholesterol accumulation within heart tissues (Han et al., 2018).

The present study showed a significant increase in MDA level and a significant decrease in TAC in the obese untreated group. Oxidative stress plays an important role in the development of comorbidities in obesity. The possible contributors to oxidative stress in obesity include hyperglycemia, elevated tissue lipid levels, vitamin and mineral deficiencies, chronic inflammation, hyperleptinemia, increased muscle activity to carry excessive weight, endothelial dysfunction, and impaired mitochondrial function (Manna and Jain, 2015). A significant positive correlation has been observed between BMI and oxidative stress biomarkers (D'Alessandro et al., 2022).

These findings are in agreement with several studies have reported lower plasma levels of FRAP and TAC in obese subjects compared to those seen in non-obese controls (Fenkeci, et al., 2003; Vincent et al., 2004). Numerous pathogenic occurrences, such as insulin resistance, diabetes, cardiovascular complications, sleep disorders, asthma, oncological issues, reproduction,

rheumatological issues, and liver failure, are brought on by obesity-induced oxidative stress. (Manna and Jain, 2015).

Through oxidation, FFAs provide the body with vital energy substrates for physiological functions; yet, elevated FFA concentrations have been shown to increase the risk of cardiovascular disease. According to reports, patients with angiographic cardiovascular disease (CVD) had higher plasma FFA levels, which were linked to the severity of their heart failure. Up to 70% of the energy needed by cardiomyocytes is produced by FFA  $\beta$ -oxidation. On the other hand, the increased cardiac FFA levels were likely to be detrimental to the heart in a number of ways. Because FFAs are amphiphilic and detergent-like, they may disrupt ion channels and membrane integrity and decouple mitochondrial enzymes, which would lower respiratory cycle efficiency and jeopardize the heart's ability to contract. (Ghosh et al., 2017).

The findings of the current study indicate that chronic exposure to a high dietary fat content can indeed induce cardiac lipid accumulation and the associated abnormalities. According to the study, 10-week-old leptin-deficient *ob/ob* mice fed conventional chow showed a three-fold rise in heart triglyceride content, relative to controls, while normal rats fed 60% HFD for six weeks had only a small elevation. (Ouwens et al., 2005). This significant increase in delivery and uptake of FAs –in addition to chronic changes in circulating hormones lead to accumulation of TAG in the obese heart. The accumulation of TAG is due to divergence of FAs uptake and oxidation. Evidence for this derived from observations that greater uptake of FAs via increased expression of the membrane FAs translocase, CD36, or reduced mitochondrial uptake and oxidation of FAs, leads to cardiac TAG, diacylglycerol (DAG) and ceramide accumulation, and contributes to a lipotoxic phenotype. The process of lipotoxicity has been recognized as an important pathological mechanism precipitating dysfunction in the obese or hypertrophied heart and is a process that contributes to heart failure (HF) in these conditions (Stanley et al., 2012). An essential stage in lipotoxicity is the shunting of activated FAs to ceramide production and altered FAs

handling in the obese and/or hypertrophied heart. Ceramide can be generated either through *de novo* synthesis requiring serine, palmitoyl-CoA and another fatty acyl-CoA species, or via recycling of more complex sphingolipid species, principally sphingomyelin (SM). Ceramides of differing chain lengths can be generated from corresponding dihydro-ceramides via the actions of dihydro-ceramide desaturase and also ceramide synthase (CerS) isoforms utilizing sphingosine and cytosolic fatty acyl Coa species leading to multiple ceramide species in the heart (Shalaby et al., 2021). Indeed, in human patients with severe HF, cardiac ceramides are increased and changes in individual serum ceramide species are observed (Ji et al., 2017). Chronic changes to cardiac ceramide species by diet may be linked with both HF incidence and progression. The metabolic alterations with increased TAG are associated with a significant increase in cardiac ceramide content which may render the heart more susceptible to apoptosis, cell loss and ultimately HF (Butler, 2017; Abdel-Baky, 2021).

The transcriptional program mediated by PPAR $\alpha$  and PPAR $\delta$  plays a central role in maintaining fatty acid oxidation in adult hearts. It is responsible for the upregulation of fatty acid oxidation during increased fatty acid supply such as fasting or eating HFD. Interestingly, sustained increases in the availability of either glucose or fatty acids result in a decrease in PPAR $\alpha$  expression (Dyck et al., 2006).

According to certain research, the heart receives more fatty acids when on a high-fat diet, and this results in an increase in PPAR $\alpha$  ligands, which activate transcription. It is plausible that elevated lipid accumulation within the TG hearts additionally induces PPAR $\alpha$  activation. (Okere et al., 2007). Hearts with elevated lipid content and inhibited fatty acid oxidation have been shown to have comparable characteristics. These findings point to a critical function of feedback loops in the network's response to metabolic stress, which is not explained by PPAR $\alpha$  overexpression or deletion. Furthermore, it's possible that the significant downregulation of PPAR $\alpha$  expression (51 percent lower) in TG hearts was a major factor in reversing the stimulatory effects of fatty acids during the HF diet, such as the up regulation of the fatty acid

transporter CD36. They suggested that this mechanism adds to the Acetyl-CoA carboxylase up-regulation and renders the TG heart resistant to HFD-induced shift to fatty acid oxidation (Yan et al., 2009).

Inducible nitric oxide synthase is an important inflammatory mediator with a vital role in immunity. However, overexpression of iNOS is hypothesized to be involved in the development of IR and CVD and may be the key link between obesity related metabolic disorders and inflammation (Jovanovic et al., 2017). In dietary and genetic models of obesity, excessive accumulation of lipids can induce iNOS expression and NO production in metabolic tissues (Puthanveetil et al., 2011). Increased cardiac iNOS expression was observed in spontaneously hypertensive rats and rabbits fed a HF-diet, as well as in obese patients (Jovanovic et al., 2017). Over-expression of cardiac iNOS in mice was reported to cause ventricular hypertrophy, which is an independent risk factor for coronary heart disease, sudden death, HF and stroke (Umar and van der Laarse, 2010). Moreover, numerous studies reported iNOS-induced myocardial dysfunction which was manifested either as a decrease in baseline myocardial contractile function, or as a reduction in  $\beta$ -adrenergic inotropic responsiveness (Puthanveetil, et al., 2011; Bai et al., 2011; Zhang et al., 2013). In addition, induction of iNOS is positively associated with the expression of CD36. This can lead to enhanced FFA uptake and TG accumulation, which can eventually cause oxidative/nitrosative stress which increase MDA levels and decrease total antioxidant capacity (Jovanovic et al., 2017)., as indicated in our study which leads to cardiac dysfunction.

Orlistat is a pharmacological agent facilitating weight loss and weight maintenance via inhibiting pancreatic lipase, an enzyme that is necessary for the triglyceride's digestion, it reduces the absorption of fat by 30%. There are no side effects of orlistat due to its lack of absorption. Orlistat act as normo-lipidemic agents that affect lipid and inhibit activation of the progenitor adipose gene induced under radiation and high-fat diet stressors and may have the potential for beneficial use on the metabolism of patients with metabolic disorders

caused by radiotherapy (Abdel-Baky& Abdel-Rahman, 2021). The current results confirmed the ameliorative role of orlistat and celery seed oil treatment alone or in combination of celery seed oil with orlistat in CVD, causing a marked lowering glucose homeostasis parameter, lipid profile, cardiac contents of triglyceride (TG), FFA, NO and ceramide. In addition, caused a down-regulation in the cardiac expression of iNOS while up-regulation of PPAR $\alpha$  expression. Our results agree with the study of Aburjai et al., 2009 who observed the hypolipidemic effects of an ethanol extract of celery seed in an obese animal model system (Aburjai et al., 2009). Celery seeds or extracts are utilized as flavorings in herbal combinations that are marketed as anti-rheumatic formulations and dietary supplements. Celery seed oil is said to include phthalate derivatives, which are responsible for the distinctive smell of celery essential oil. Moreover, drinking aqueous celery extract for 8 weeks significantly reduced the serum TC concentration by enhancing the excretion of TC metabolites via bile acids and faces because bile acid excretion represents the major pathway for TC degradation and removal from the body. Furthermore, b-pinene and g-terpene are flavor compounds in celery oil. Terpenoids can be described as chemically modified terpenes. Daily eating of certain terpenoids of plant origin might be useful for the management of obesity-induced metabolic disorders, such as type 2 diabetes, hyperlipidemia, CVD and lower prevalence of metabolic syndrome (El-Shinnawy, 2015). Celery, because of compounds such as caffeic acid, *p*-coumaric acid, ferulic acid, apigenin, luteolin, tannin, saponin, and kaempferol has powerful antioxidant characteristics (Kooti and Daraei, 2017). As shown in our study, celery seed oil is indicated by significant reduction in MDA, NO content and a significant increase in TAC of cardiac tissue. Celery seed oil inhibits adipocyte differentiation through the downregulation of adipogenic genes such as Fatty acid synthase (FAS). In addition, celery oil suppresses the production of lipids and the synthesis of adipocyte hormones (leptin and adiponectin) that play an important biochemical role in adipogenesis (Kim et al., 2021). As shown in

our result, celery seed oil significantly reduced cardiac TG and FFA content. The anti-lipogenic and antioxidant effect of celery seed oil stimulate the reduction of ceramide content, iNOS expression and increase the PPAR $\alpha$  expression, as showed a positive correlation between ceramide content, iNOS expression with TG, FFA and MDA content, and a negative correlation between PPAR $\alpha$  expression.

### Conclusion

From the results of the present study and the above-mentioned discussion, we can confirm the anti-obesity potential of celery seed oil and orlistat in HFD-rat model of obesity and also their beneficial effects for protection against the complication of obesity. celery seed oil has a powerful glucose and lipid-lowering effect that plays an important role in protection against obesity. Celery seed oil has an ameliorative effect against high-fat diet-induced lipotoxic heart disease, through multiple interrelated mechanisms including, ameliorated the histological changes, boosting the antioxidant status, suppressing the TG and FFA accumulation in cardiac tissue. The used dose of celery seed oil produces anti-lipotoxic effects equivalent to orlistat, while the combined group which was treated with orlistat and celery seed oil showed the best effect. These effects may be mediated through up-regulation of *PPAR- $\alpha$*  and suppression of iNOS. In addition, celery seed oil can be used as adjuvant with orlistat drugs to enhance their pharmacological effect against lipotoxicity of heart tissues.

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Not applicable.

### Conflicts of interest

There are no conflicts of interest to declare.

### Author Contributions:

Shrouq A. Salem: Performed practical work, acquisition of data, data analysis and interpretation, and writing the manuscript. Suzan M. Abdel-Tawab: Proposed research plan, analyzed and interpreted the results, edited and reviewed the manuscript. Bothaina F. Mahmoud: Supervised the practical part, data analysis and interpretation, and writing of the manuscript. Sara A. Shaker: Supervised the practical part, data analysis and interpretation,

and writing of the manuscript. All authors read and approved the final manuscript.

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