



## Antiobesity activity and hypolipidemic effect of Proanthocyanidins in rats fed a high fat diet

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### ABSTRACT

Proanthocyanidins, also known as condensed tannins, can prevent obesity induced oxidative stress damage in the liver. This study aimed to evaluate the protective effect of Proanthocyanidins on obesity induced oxidative stress and biochemical abnormalities in male rats. Forty-eight male albino rats were divided into three equal groups. Group I (control normal): rats received normal diet. Group II (Obesity): rats received high fat diet (HFD). Group III (Obesity+ Proanthocyanidins): rats received Proanthocyanidins (50 mg/kg.b.wt.) orally for two months after induction of obesity. The obtained results showed significant increase in serum insulin, lipids profile (total cholesterol and triacylglycerols) concentrations, liver L-MDA in addition to up regulation of leptin and Nuclear factor kappa B (NF- $\kappa$ B) gene expression level in liver tissues of obese rats. However, liver catalase activity and GSH concentration were markedly decreased. These results suggested that, Proanthocyanidins treatment exerts a protective effect on obesity by reduction of oxidative stress, inflammation and hyperlipidemia in rats through free radical scavenging and anti-inflammatory activities as well as regenerating endogenous antioxidant defense system mechanisms.

**Keywords:** Obesity, Proanthocyanidins, oxidative stress, insulin, leptin

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### 1. INTRODUCTION

Obesity is the excessive fat accumulation in the body, chronic and nutritional disorder and insulin resistance metabolic syndrome (You *et al.*, 2014) which indirectly reduced life quality and cause mortality and morbidity (Cherniack, 2008). Obesity has been officially recognized as an independent disease entity by the American Medical Association. The World Health Organization (WHO) has classified obesity as one of the leading risk factors for global deaths due to its tight association with

multiple chronic diseases, such as cardiovascular disease, type II diabetes, and certain forms of cancers (Haslam and James, 2005). There are many different options for obesity treatments, including dietary control, exercise, life-style changes, prescription weight-loss medications, and weight-loss surgeries (Chandrasekaran *et al.*, 2012).

Proanthocyanidins are a class of flavonoid compounds that are present in many food and drinks such as fruits, vegetables, and red wine, and thus they are very abundant in the human diet, have been defined as healthy

bioactive compounds (Blade *et al.*, 2016). Several studies have reported many beneficial effects of (GSPE) on various obesity associated diseases, such as insulin resistance, (Montagut *et al.*, 2010) dyslipidaemia, (Quesada *et al.*, 2009) hypertension, (Pons *et al.*, 2014) inflammation (Martinez-Micaelo *et al.*, 2012) and leptin resistance (Ibars *et al.*, 2017).

Proanthocyanidins (PA) is structurally diverse, including with regard to the number of monomer units involved. The PA that consist exclusively of epicatechin and catechin units are called procyanidins and these are the most abundant type of PA in plants. Grape seed extract (GSE) is a good source of PA containing abundant oligomers and polymers of monomeric units of (-)-epicatechin or (+)-catechins, including the gallated forms the ability of a compound to act as an antioxidant depends highly on its chemical structure (Manach *et al.*, 1996). There are studies with different animal models and with PA rich extracts from different sources, indicating that PA reduces fat and body weight. In fact, PA appears to reduce the body weight gain rather than to reduce the body weight (Pajuelo *et al.*, 2012). Furthermore, proanthocyanidins is a potent scavenger of peroxy and hydroxyl radicals that is generated in the reperfusion myocardium after ischemia (Pataki *et al.*, 2002). In addition to the ability to scavenge ROS, GSP has the ability to stimulate NO production in a dose dependent manner, which is a relatively stable free radical and acts as a signaling molecule in diverse physiological and pathological pathways.

This study was to investigate the possible beneficial effect of Proanthocyanidins against deleterious effect of obesity in adult male rats through investigation of insulin, lipid profile, inflammatory markers, biomarkers of

oxidative stress.

## 2. MATERIALS AND METHODS

### *Experimental animals:*

Forty-eighth white male albino rats of 12-16 weeks old age and average body weight 160-200 g were used in this study. Rats were housed in separated metal cages and kept at constant environmental and nutritional conditions throughout the period of experiment. Rats were fed on constant ration and fresh, clean drinking water was supplied ad-libitum. All rats were acclimatized for minimum period of 15 days prior to the beginning of study.

### *Ration and additives:*

There are two type of ration were prepared freshly and daily throughout the course of experiment: a standard diet with protein 20%; fat 5%;carbohydrates 5%; fiber 5% and a high-fat diet with 20% of energy derived from protein;15% from corn oil; 50% from sucrose; 5% from fiber (Surapaneni and Jainu, 2014).

### *Induction of obesity:*

The experimental induction of obesity in male rats was induced by feeding the rats on the prepared high fat diet (HFD) for eight weeks before the beginning of the experiment. The diet was prepared, and necessary vitamins and minerals were added. For fatty diet the chow, in powder form, was mixed fat until become homogenous in a dough-like consistency. This dough was shaped with a paste injector. The obtained chow blocks were dried and used for rats feeding for two months. After two months of obesity induction treatment with Proanthocyanidins were given and continued for additional two months.

### *Chemicals and antioxidant:*

The antioxidant and chemicals used in the present study were:

a.Proanthocyanidins was provided by El-

Debeky Co. For Trading Chemicals, Medicines And Medical Appliances, Egypt.

b- Dimethyl sulfoxide (DMSO) (100%): Proanthocyanidins was dissolved in DMSO and further diluted with normal saline. The final concentrations of DMSO in solution were <1%. The freshly prepared solution of Proanthocyanidins was administered orally and daily at a dose of 50 mg/kg. body weight for group III for two months (Gil Cardoso *et al.*, 2017).

c- Other chemicals used in this study were of the highest purified grades available purchased from El Gomhouria Company for Trading Chemicals and Medical Appliances, Egypt.

#### *Experimental design:*

After acclimatization to the laboratory conditions, the animals were randomly divided into three groups (10 rats each) placed in individual cages and classified as follow:

Group I (normal control group): Rats received normal diet, served as control non- treated for all experimental groups.

Group II (Obesity group): Rats received high fat diet (HFD) ad-libitum, served as obesity induced rats group.

Group III (Obesity+proanthocyanidins treated group) rats received proanthocyanidins orally at a dose of (50 mg/kg.b.wt./day) for 2 months after induction of obesity.

#### *Sampling:*

Blood samples and liver tissue specimen were collected from all animal groups (control and experimental groups) once after the end of 4 months.

#### *Blood samples:*

Blood samples were collected by ocular vein puncture from all animal groups after overnight fasting in dry, clean tubes and allowed to clot for 30 minutes and serum was separated by centrifugation at 3000 r.p.m for 15 minute. The serum was taken by automatic

pipette and received in dry sterile tubes, then kept in deep freeze at -20 °C until use for subsequent biochemical analysis. All sera were analyzed for determination of the following parameters: Insulin, Total cholesterol and Triacylglycerols.

#### *Tissue samples:*

##### *Liver tissue for biochemical analysis:*

About 0.5 g of liver tissue specimen was taken from each group of rats after had been euthanized. The specimens were immediately removed and washed several times with saline and blotted between two damp filter papers, weighed and stored at -20°C for subsequent biochemical analysis.

##### *Preparation of liver tissue homogenate:*

Briefly, liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10 % homogenates. The homogenates were centrifuged at 6000 r.p.m for 15 minutes at 4°C then the resultant supernatant was used for the determination of the following parameters: L-MDA and Catalase.

About 0.2 g of liver tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1 hour and centrifuged for 10 min at 3,000 r.p.m then the clear supernatant was removed and used for determination of GSH concentration.

##### *Liver tissue for molecular analysis:*

About 0.5 g of liver tissue put in eppendorf tubes and were immediately kept in liquid nitrogen and stored at -80°C till RNA extraction for determination of Leptin and NF-κB gene expression level.

*Biochemical analysis*

Serum Insulin was determined according to the method described by Wilson and miles, (1977). Total cholesterol and Triacylglycerols were determined according to the method described by NCEP expert panel, (1988) and Stein, (1987), respectively. Liver tissue L-MDA, CAT and GSH were determined according to the method described by Mesbah *et al.*, (2004), Luck, (1974) and Moron *et al.*, (1979).

*Molecular analysis*

Total RNA was isolated from liver tissue of rats using RNeasy Mini Kit (Thermo Qiagen, #74104) according to the manufacturer’s protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA), 5 mg of total RNA

from each sample was reverse transcribed using Quantiscript reverse transcriptase. The produced cDNA was used as a template to determine the relative expression of Leptin and NF-κB genes using Step One Plus real time PCR system (Applied Biosystem, USA) and gene specific primers. The reference gene, βactin, was used to calculate fold change in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression was done. For the treated groups, assessment of  $2^{-\Delta\Delta Ct}$  determined the fold change in gene expression relative to the control (Livak and Schmittgen, 2001).

Forward and reverse primers sequence for real time PCR.

Gene	Forward primer (5' ----- 3')	Reverse primer (5' ----- 3')
Leptin	GACATTTACACACGCAGTC	GAGGAGGTCTCGCAGGTT
NF-κB	CCTAGCTTTCTCTGAACTGCAA	GGGTCAGAGGCCAATAGAGA
β-actin	ACCCACACTGTGCCCATCTA	CGTCACACTTCATGATG

*Statistical analysis:*

The results were expressed as mean ± SE using SPSS (13.0 software, 2009) program. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity. Values were considered statistically significant when p<0.05.

**3. RESULTS:**

The data presented in table (1) showed a significant increase in serum insulin, total cholesterol and Triacylglycerol concentrations in obesity-induced rats when compared to normal control group. However,

Proanthocyanidins treatment to obese male rats caused a significant decrease in elevated serum insulin, total cholesterol and Triacylglycerol concentrations when compared with obesity induced group.

The obtained results presented in table (2) revealed that, obese rats showed significant increase in liver tissue L-MDA and significant decrease in Enzymatic antioxidant catalase and Non-enzymatic antioxidant reduced glutathione (GSH) when compared with normal control group. On the other hand, Proanthocyanidins treatment to obese male rats caused a significant decrease in liver tissue L-MDA with marked increase in catalase activity and GSH concentration when compared with in obesity-induced rats.

The qPCR results presented in table (3) showed a significant up-regulation in relative expression of Leptin and NF- $\kappa$ B gene level in liver tissue of obesity induced rats when compared to normal control group.

However, Proanthocyanidins treatment to obese rats caused a significant down-regulation in Leptin and NF- $\kappa$ B gene expression level when compared with obesity-induced rats.

Table (1): Effect of Proanthocyanidin administration on serum insulin, total cholesterol and triacylglycerols concentrations in obesity induced in male rats.

Parameters	Insulin (ng/ml)	Total Cholesterol (mg/dl)	Triacylglycerols (mg/dl)
Exp. groups			
Group I: Normal control	4.68 $\pm$ 0.21 <sup>c</sup>	80.21 $\pm$ 2.53 <sup>c</sup>	57.15 $\pm$ 2.83 <sup>c</sup>
Group II : (Obese)	9.82 $\pm$ 0.37 <sup>a</sup>	155.50 $\pm$ 3.85 <sup>a</sup>	113.00 $\pm$ 4.63 <sup>a</sup>
Group III: Obese + Proanthocyanidin	7.45 $\pm$ 0.31 <sup>b</sup>	99.30 $\pm$ 2.45 <sup>b</sup>	83.00 $\pm$ 3.04 <sup>b</sup>

Data are presented as (Mean  $\pm$  S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P $\leq$ 0.05).

Table (2): Effect of Proanthocyanidin administration on liver tissue L-MDA, Catalase and GSH in obesity induced in male rats.

Parameters	L-MDA (mmol/ g tissue)	Catalase (ng/g.tissue)	GSH (ng/g.tissue)
Exp. groups			
Group I: Normal control	11.14 $\pm$ 0.52 <sup>d</sup>	6.72 $\pm$ 0.38 <sup>a</sup>	113.07 $\pm$ 4.15 <sup>a</sup>
Group II : (Obese)	25.60 $\pm$ 1.01 <sup>a</sup>	2.10 $\pm$ 0.15 <sup>c</sup>	47.23 $\pm$ 1.82 <sup>d</sup>
Group III: Obese + Proanthocyanidin	19.90 $\pm$ 0.34 <sup>b</sup>	4.47 $\pm$ 0.42 <sup>b</sup>	96.21 $\pm$ 2.42 <sup>b</sup>

Data are presented as (Mean  $\pm$  S.E). S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P $\leq$ 0.05).

Table (3): Effect of Proanthocyanidin administration on relative expression of Leptin and NF- $\kappa$ B gene in liver of obesity induced in male rats.

Parameters	Leptin		NF- $\kappa$ B	
	Fold change mean	SEM	Fold change mean	SEM
Exp. groups				
Group I: Normal control	1.00 <sup>d</sup>	0.06	1.00 <sup>d</sup>	0.05
Group II : (Obese)	10.20 <sup>a</sup>	0.34	5.70 <sup>a</sup>	0.27
Group III: Obese+Moringa Oleifera	4.23 <sup>c</sup>	0.19	3.71 <sup>b</sup>	0.21

Means within the same column carrying different superscript letters are significantly different ( $P \leq 0.05$ ).

#### 4. DISCUSSION

Cardiovascular disease (CVD), also known as heart and blood vessel disease, is the leading cause of death (Cook and Cantor, 2006). In most cases, these clinical conditions result from atherosclerosis, which was once identified as a lipid-storage disease. Obesity results from an imbalance of energy homeostasis, over an extended period of time, caused by the consumption of more calories than the body is able to burn. The cause of this imbalance is complex and is influenced by the convergence of various environmental, behavioral and genetic factors (Bray, 2004).

The obtained results revealed that, obesity-induced rats showed significant increase in serum insulin, total cholesterol and triacylglycerols concentrations when compared to normal control group. These results were nearly similar to (Woods *et al.*, 2004) who recorded that, hyperinsulinemia and insulin resistance are induced by high-fat feeding. Also, Cyrus *et al.*, (2003) showed that, mice fed HFD had a significant increase in both cholesterol and triglycerides, the higher concentrations of such lipid profile in the high fat fed rat lead to the development of abnormal lipid metabolism and atherosclerosis (Taboada *et al.*, 2006). Insulin resistance in obesity is also characterized by

chronic low-grade inflammation and reduced plasma levels of adiponectin, an adipokine that improves insulin sensitivity. Along with increased plasma levels of free fatty acids, hyperglycemia, and reactive oxygen species, these factors can change gene expression and cell signaling in vascular endothelium which also alters the release of endothelial vasoactive factors (Rask-Madsen and King, 2007). Loss of insulin action causes a shift in balance from oxidation to esterification of free fatty acids (FFAs), resulting in elevated very low-density lipoprotein (VLDL) secretion. Also, insulin resistance can be considered as additional factor which contributes to increased cardiovascular disease in obesity. The action of insulin is initiated by binding to its receptors and activation of intrinsic protein tyrosine kinase activity of the receptors, resulting in initiation of intracellular signaling cascade that eventually related to glucose and lipid metabolism (Westerbacka *et al.*, 2002). It is well established that increased availability and utilization of free fatty acids (FFAs) play a critical role in the development of insulin resistance. Excess adipose tissue has been shown to release an increased amount of FFAs which directly affect insulin signaling, diminish glucose uptake in muscles, drive

exaggerated triglyceride synthesis and induce gluconeogenesis in the liver leading to elevated levels of glucose and lipids (Mlinar *et al.*, 2007). In general, the development and progression of atherosclerosis is considered to be a form of chronic inflammation (Ross *et al.*, 1999). Endothelial dysfunction is the key process promoting inflammatory reactions (Wang *et al.*, 2000).

Treatment with Proanthocyanidins to obese male rats caused a significant decrease in serum insulin, total cholesterol and triacylglycerol concentrations. These results were nearly similar to those recorded by Dorenkott *et al.*, (2014) who found that, in mice fed a HFD, supplements consisting of proanthocyanidins prevented weight gain, impairment of glucose tolerance and insulin resistance. Also, Pallares *et al.*, (2013) found that, chronic corrective treatment using proanthocyanidins (PA) ameliorates insulin in rats fed a cafeteria diet (CD). The effects of this extract might be partially explained by its insulin-like effect on insulin-sensitive cell lines, as well as through its effects on pancreatic  $\beta$ -cell function and by preventing the effects of the CD on insulin secretion and production and lipid accumulation in the pancreas. The antihyperglycaemic effect of Grape seed proanthocyanidins extract (GSPE) may be partially due to the insulinomimetic activity of the procyanidins using insulin-sensitive cell lines (Pallares *et al.*, 2013). PA act as a hypolipidemic agent (Arola *et al.*, 2010), and improve inflammation (Terra *et al.*, 2011) and insulin resistance. Pons *et al.*, (2017) reported that, the daily administration of GSPE (at doses of 25 mg/kg per day, 100 mg/kg per day, and 200 mg/kg per day) for 12 weeks together with a diet high in carbohydrates and fat in rats can mitigate the increase in blood pressure and plasma lipids levels in response to the obesogenic diet. The

proanthocyanidin hypotriglyceridemic protective effect is in concordance with the preventive decrease in adipocyte hypertrophy in retroperitoneal white adipose tissue (rWAT), since visceral adipocyte hypertrophy has been associated with dyslipidemia (Veilleux *et al.*, 2011).

The levels of the TG-rich lipoproteins, chylomicrons (CM) and very low-density lipoproteins (VLDL) depend on the secretion of these lipoprotein complexes by the intestine and the liver and on their catabolism by the lipoprotein lipase (LPL) in extra-hepatic tissues. Interestingly, PA represses the secretion of VLDL-TG and CM-TG. Without affecting LPL activity or expression in WAT and muscle (Quesada *et al.*, 2012). Therefore, it is possible that the PA reduce plasma TG by repressing lipoprotein secretion and not by affecting its catabolism. Impaired lipid availability in enterocytes seems to be the primary mechanism for the PA-induced reduction of CM secretion. In this context, PA increase the faecal excretion of cholesterol in rats and inhibit the activity of pancreatic lipase *in vitro*, (Moreno *et al.*, 2003) indicating reduced lipid absorption. Moreover, PA represses the expression of long chain acyl-CoA synthetases (ACSL)-5 and -3, which supply fatty acids for TG synthesis in enterocytes. Thus, the results of this study are agreement with the well-defined hypolipidemic and cardioprotective effect of proanthocyanidins.

The presented findings showed that, obese rats group showed significant increase in liver tissue L-MDA and markedly decreased catalase activity and GSH level when compared to control group. Similarly, Balkan *et al.*, (2002) reported that, high-cholesterol (HC) diet had an increasing effect on lipid peroxidation in plasma and tissue in rabbits. High fat diet (HFD) has a role in

generating oxidative stress, which results from an imbalance between the production of free radicals, and the scavenger antioxidant system (Balkan *et al.*, 2004). Obesity is also associated with oxidative stress, which may be due to extended postprandial hyperlipidemia and/or hyperglycemia. Obese humans have increased levels of oxidative stress and this is ameliorated by diet restriction and weight loss (Dandona *et al.*, 2001). Also, obesity is associated with increased activities of the secondary product of lipid peroxidation, and weight loss has positive effects on oxidative stress and antioxidant activity (Uzun *et al.*, 2004). Moreover, increased lipid profile could also contribute to increased oxidative stress in obesity, where increased lipid substrate in the tissues may increase the mechanical and metabolic load on such tissues, thus increasing oxygen consumption. A negative consequence of the elevated oxygen consumption is the production of reactive oxygen species (ROS) (Vaz *et al.*, 1997). Lipid peroxidation is a marker of cellular damage initiated by ROS. In obesity, lipid peroxidation is thought to play a role in the etiology of existing health problems, such as cardiovascular disorders. Increased lipid peroxidation is considered responsible for impairment of endothelial cells, capillary permeability and vascular integrity (Keidar *et al.*, 2004). Further increase in superoxides, H<sub>2</sub>O<sub>2</sub>, hydroxyl radicals and lipid peroxidation consequently cause decrease in the effectiveness of antioxidant defenses, like SOD, CAT and GPx (Hafize *et al.*, 2007).

Treatment with Proanthocyanidins to obese rats caused a significant decrease in liver tissue L-MDA and marked increase in catalase activity and GSH level when compared to obesity non treated group. These results were nearly similar to Song *et al.*, (2012) who suggested that, the protective

effects of PA against obesity and hepatic ischemic reperfusion injury (IRI) were related with their roles in reducing the tissue oxidative stress level as GSP 20 mg/kg/day for 3 weeks decrease oxidative stress by reducing MDA content and elevating of superoxide dismutase (SOD), (CAT), glutathione peroxidase (GSH-PX), total nitric oxide synthase (T-NOS) and iNOS in high-fat diet induced obese mice. As reactive oxygen species (ROS) play important part in obesity and hepatic IRI, GSP have potential protective effects against obesity and hepatic IRI as showed its ideally protective effects on curbing the obesity on mice, reducing hepatic fat content and against liver IRI: particularly in high-fat diet induced obese mice. Also, Fernandez-Iglesias *et al.*, (2013) demonstrated that, GSPE administration improves the liver GSH metabolism, and in consequence, increases the total antioxidant capacity of the cell and improve the antioxidant enzymes activity which indicated an improvement in the redox homeostasis by the polyphenol content in GSPE in a rat model of genetic obesity which might be due to the oxidative stress-reducing capacity and inhibition of the generation of superoxide anions and hydroxyl free radicals (Shrivastava *et al.*, 2013). In view of its ability to scavenge ROS, PA can stabilize and inactivate free radicals by donating an electron to free radical -OH groups attached to the phenolic ring, which helps to terminate oxidative chain reactions.

The Presented data exhibited that, obese rats showed significant up-regulation of Leptin and NF- $\kappa$ B gene expression level in liver tissue when compared to normal control group. These results were nearly similar to Tulipano *et al.*, (2004) who showed that, leptin level was higher in adult male Sprague-Dawley rats fed a high-fat diet (31% of energy) for one week. Most obese individuals



have high concentrations of leptin but exhibit leptin resistance because of decreased leptin transport into the central nervous system or down regulation of leptin receptors (Bjorbaek *et al.*, 1999). Leptin communicates the amount of stored energy to the brain and activates the hypothalamic center which regulates energy intake and in turn body energy expenditure (Metwally *et al.*, 2017). The amount of stored triglycerides in the adipose tissue correlates to the expression of leptin and circulating leptin concentrations (Considine *et al.*, 1996). There is provide evidence that, leptin was elevated in obese human (Orel *et al.*, 2004) and animals (Scarpace and Zhang, 2008). Moreover, Masoud and Adel, (2006) reported that, serum leptin concentration was increased in relation to increased body fat content. The positive correlation between body fat and serum leptin is probably explained by the increased release of leptin from large fat cells. Thus, leptin can serve as an indicator of fat content and its level may be decreased by reduction of body weight. Additionally, Lin *et al.*, (2000) suggested that, during high fat feeding animals are sensitive to the food lowering effect of leptin. However, despite the reduction in food intake, animals become fat as a result of the increase in food efficiency leading to an increase in leptin level followed by resistance to its action. Leptin is a cytokine like polypeptide produced by the adipocytes and it is overproduced during obesity due to the generation of ROS (Assal *et al.*, 2007). Inflammation is a host defense mechanism to protect against pathogens, stresses and tissue damage, and is a major factor in the progression of many chronic diseases including ulcerative colitis, diabetes, atherosclerosis, obesity and arthritis (Nathan, 2002). The inflammatory response to foreign pathogens and general stressful insults involves a combination of different signaling

elements such as cytokines, nitric oxide (NO) and two key transcription factors, nuclear factor-kappa B (NF- $\kappa$ B) and nuclear factor (erythroid- derived 2)-like2 (Nrf2) is activated in most cell types as mice-fed HFD displayed about 3.5-fold increased whole body NF- $\kappa$ B activity (Wardyn *et al.*, 2015). Additionally, De Souza *et al.*, (2008) reported that, NF- $\kappa$ B is increased about twofold in the liver, hypothalamus and skin of rodents fed with HFD for 6 months compared with animals fed with a control diet. NF- $\kappa$ B was similarly elevated in the liver and skin in common genetic obesity models of genetic hyperphagia (ob/ob mice and fa/fa rats) (Katiyar and Meeran, 2007).

Treatment with Proanthocyanidins to obese rats caused a significant down-regulation of Leptin and NF- $\kappa$ B gene expression level in liver tissue when compared to obesity non treated group. These results are nearly similar to those recorded by Boque *et al.*, (2012) who reported that, supplementation of high-fat-sucrose diet with proanthocyanidins during 8 weeks (700mg/Kg body weight) prevented body weight and adiposity gain promoted by this obesogenic diet, and decrease leptin level without changes in food intake. Results of leptin gene expression are in line with the decreased circulating leptin levels and reflect the reduced fat mass observed in proanthocyanidins - supplemented animals. The NF- $\kappa$ B complex is a heterodimer of two subunits, p50 and p65, which exist in the cytoplasm in an inactive form, and it is related to the inhibitory subunit, inhibitor protein  $\kappa$ B- $\alpha$  (I $\kappa$ B- $\alpha$ ). inflammation and ROS stimulate NF- $\kappa$ B activation by enhancing the dissociation of cytoplasmic NF- $\kappa$ B from I $\kappa$ B- $\alpha$ , thereby allowing NF- $\kappa$ B to migrate to the nucleus (Noh *et al.*, 2006), where it binds to promoters of NF- $\kappa$ B-regulated genes to initiate gene transcription (Li and Verma, 2002). Yokozawa *et al.*,

(2012) Reported that, a significant increase in the NF- $\kappa$ Bp65 protein level and significant reduction of the I $\kappa$ B- $\alpha$  protein level were observed in the diabetic rat. However the administration of proanthocyanidin led to a decrease in NF- $\kappa$ Bp65 and elevation of I $\kappa$ B- $\alpha$  protein, indicating that proanthocyanidin suppressed the translocation of NF- $\kappa$ B to the nucleus, where it binds to the promoters of NF- $\kappa$ B-regulated genes and initiates gene transcription. Terra *et al.*, (2007) found that, proanthocyanidins treatment decreased NF- $\kappa$ B in liver, which is directly associated with the decreased hepatic expression of such inflammatory molecules as TNF- $\alpha$  and C-reactive protein (CRP). Proanthocyanidins also reduce Monocyte chemoattractant protein-1 (MCP-1) secretion in adipocytes partially due to the diminished TNF- $\alpha$  levels that we have detected in adipose tissue, muscle and liver, which might be a consequence of the inhibitory effects of proanthocyanidins on NF- $\kappa$ B activation. Also, Kim *et al.*, (2018) showed that, GSPE treatment diminishes the phosphorylation of I $\kappa$ B $\alpha$  and inhibits nuclear translocation of p65 and p50 NF- $\kappa$ B subunits. The results indicate that GSPE inhibits NF- $\kappa$ B activation through the suppression of I $\kappa$ B $\alpha$  phosphorylation as PA is a strong antioxidant and anti-inflammatory agent.

## 5. Conclusion:

The present study demonstrated that, administration of Proanthocyanidins improve the harmful effects caused by high fat diet induced obesity. Obesity exaggerated different organs mainly liver and these occurred through changes in several parameters. high fat diet induced obesity caused significant increase in serum insulin, total cholesterol, triacylglycerols and liver tissue L- MDA, Leptin and NF- $\kappa$ B. However, a significant reduce in liver tissue catalase and GSH were shown in obese rats. Proanthocyanidins

treatment in obese rats relieved all aforementioned parameters near its normal range with best results after 8 weeks. So, these effects confirmed the strong antioxidant, anti-inflammatory properties of Proanthocyanidins in obesity.

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