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POSSIBLE MITIGATING EFFECTS OF CALORIC RESTRICTION OR PORTULACA OLERACEA L. (PURSLANE) EXTRACT ON COAGULATION AND FIBRINOLYTIC PROCESSES OF OBESE RAT

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Abstract

Obesity and so metabolic syndrome predispose to activate coagulation factors causing prothrombotic state, while restoring normal body weight is an appropriate strategy for controlling thrombosis. The aim of this study was to assess the role of induced obesity then weight reduction by caloric restriction or purslane extract on coagulation and fibrinolytic process in rats. Adult male rats weighing from 155-185 g were divided into 5 groups. 1st fed on normal diet. 2nd fed on HFD for 18 weeks. 3rd fed on HFD for 18 weeks then diet restriction for 4 weeks. 4th fed on HFD for 18 weeks then diet restriction for 8 weeks. 5th fed on HFD and 1 g/ kg b.w. of purslane extract for 18 weeks. In the present study, induced obesity resulted in an increase in the final body weight, and in the levels of serum insulin, leptin, blood glucose, HOMA-IR, TC, TG, LDL-c, VLDL-c, fibrinogen, factors (V, VII, X) %, vWF %, D-dimmers and PAI-1levels. while showed a decrease in the levels of HDL-c, CT, PT, aPTT whereas, restoring normal body weight by caloric restriction (4 and 8weeks) or purslane extract in 3rd, 4th and 5th groups respectively for an extent, mitigate the disturbance of these parameters. In conclusion, HFD-induced obesity in rats is associated with hypercoagulability and impairs fibrinolytic activity that could be greatly reversed by caloric restriction or oral administration of purslane extract.

Abbreviations
aPTT: activated partial thromboplastin time,
BMI: body mass index,
HDL: high density lipoprotein,
HFD: high fat diet,
HOMA- IR: Homeostasis model assessment of insulin resistance,
IL-1*β*: interleukin-1*β*,
IL-6: interleukin-6,
LDL: low density lipoprotein,
NO: nitric oxide,
PAI-1: plasminogen activator inhibitor-1,
PT: prothrombin time,
Rest -4: four weeks diet restriction,
Rest -8: eight weeks diet restriction,
TNF-*α*: tumor necrosis factor-*α*,
VLDL: very low density lipoprotein

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I. INTRODUCTION

overweight and obesity belong to the group of chronic diseases in which there is a substantially increased risk of haemostatic disorders, including excessive activation of coagulation system, inhibition of fibrinolysis, decreased endothelial thrombo- resistance and/or pro-inflammatory state **(Duburcq *et al*., 2014; Kornblith *et al*., 2015 and Ohkura *et al*., 2015)** . Obesity also plays a central role in the development of metabolic syndrome, a cluster of cardiovascular risk factors, which leads to an approximately three fold risk to coronary heart disease and cerebrovascular arterial stroke **(Klein *et al*., 2004 and Sen *et al*., 2008)**.

Adipose tissue play a causal role in the prothrombotic state observed in obesity, by affecting haemostasis, coagulation and fibrinolysis **(Kim *et al*., 2016)**. Adipose tissue induces thrombocyte activation by the production of adipose tissue-derived hormones, often called adipokines, of which some such as leptin and adiponectin have been shown to directly interfere with platelet function. Increased adipose tissue mass induces insulin resistance (IR), systemic low-grade inflammation, and affecting platelet function **(Rabe *et al*., 2008)**. Also, adipose tissue directly impairs fibrinolysis by the production of plasminogen activator inhibitor-1(PAI-1) and possibly thrombin-activatable fibrinolysis inhibitor. It contributes to enhanced coagulation by direct tissue factor production, but hypercoagulability is likely to be primarily caused by affecting hepatic synthesis of the coagulation factors fibrinogen, factor VII, factor VIII and tissue factor, by releasing free fatty acids and pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) into the portal circulation and by inducing hepatic IR **(Faber *et al*., 2009)**.

It is known that, the biological functions of adipokines are still partially unknown; however, they seem involved in the regulation of many physiological processes, such as appetite regulation and energy balance, lipid metabolism, blood pressure, insulin sensitivity, inflammation, haemostasis and angiogenesis **(Attie & Scherer, 2009 and Wang & Nakayama, 2010)**. In the plasma of patients with obesity or metabolic syndrome, increased levels of some adipokines can be measured, suggesting that these adipocyte-derived substances might be considered as novel biomarkers and regulators of the metabolic syndrome **(Deng and Scherer, 2010)**.

The pro-thrombotic actions of leptin *in vivo* are related to an influence on platelet function, and on coagulation/fibrinolysis balance, resulting in enhanced agonist-induced platelet aggregation and increased stability of arterial thrombi **(Corsonello *et al*., 2003).**

It was also shown that weight loss and exercise could ameliorate the inflammatory milieu of patients with metabolic syndrome by modulating their adipokine profile **(Kadoglou *et al*., 2007 and Deng & Scherer, 2010, Korybalska *et al*., 2016)**.

PAI-1 inhibits tissue plasminogen activator, which cleaves plasmin from plasminogen and is therefore, the primary physiological inhibitor of fibrinolysis *in vivo* **(Kershaw & Flier, 2004**). Elevated PAI-1 levels compromise the normal clearance of fibrin and consequently promote thrombosis. PAI-1 levels are positively correlated with obesity, insulin resistance, and triglyceride levels, and PAI-1 levels are significantly reduced by weight loss in obese individuals **(Orenes-Piñero *et al*., 2015 and Perkins *et al*., 2015)**. The increased levels of PAI-1 found in obesity may predispose to micro- and macro-vascular, arterial and venous, thrombosis **(Kershaw & Flier, 2004)**.

Purslane is one of wild plants which are used as nutritional food, It is an annual green herb with edible succulent stems and leaves, slightly acidic and spinach-like taste. The species name *oleracea* originates from Latin, meaning pertaining to kitchen garden referring to its use as a vegetable **(Boulos and El-Hadidi, 1984).** It contain β-carotene and fatty acids (linolenic acid, alpha linolenic acid, palmitic acid, stearic acid**,** oleic acid**),** omega 3 and 6 and higher amounts of vitamin E&C than cultivated plants **(Abd El-Latif, 2008 and Oliveira et al., 2009).** Moreover, this wild plant is rich in flavonoids, alkaloids and other phenolic compounds which increase its antioxidant capacity **(Xiang *et al*., 2005, Xu *et al.*, 2006 and Lim & Qual, 2007)**.

The antioxidant and nitric oxide (NO) inhibition activities of methanolic extract of *P. oleracea L* was investigated. It was shown that, *P. oleracea* has high level of antioxidant activity compared with α-tocopherol. It has moderate

free radical scavenging activities. It showed inhibitory effect on lipopolysaccharide and interferon-γ- induced NO production **(Abas *et al.*, 2006)**. The effect of *P. oleracea* seeds crude lipids at dose 1g/kg/day orally for 60 days as hypolipidemic on rats was investigated. Body weight gain and mean food consumption was decreased compared to hyperlipidemic control. Serum triglycerides, total lipids, total cholesterol and LDL cholesterol were declined also, while HDL-cholesterol was increased significantly **(Abd El-Latif, 2008, Sadeghi *et al.*, 2016)**.

So the present study was designed to investigate the effects of diet-induced obesity and weight reduction by caloric restriction or purslane extract on blood coagulability and fibrinolytic activity in male rats.

II. Materials & methods

1. Experimental Animals

The practical part of this study was carried out at research laboratory of Physiology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt. Fifty adult male rats were used throughout this study (weighing 155-185 g.) They were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University. The animals were housed under hygienic conditions in 10 steel wire cages (5 rats / cage). Animals were allowed free access to diet and water in good air conditioned room for two weeks before starting the experiment. We have followed the European committee directive and national rules on animal care.

2. Purslane Preparation

purslane was collected from corn fields of farmers around Zagazig city, dried in shade under laboratory conditions for 15 days, transferred to oven at 60 C for 48 h., then grinded into a finely powdered material. The powder was extracted with distilled water in water bath at 80 C, then extracted by maceration in room temperature 5 times, each time one week by 70% ethanol. The total extract was concentrated under reduced pressure. 200 g. of purslane extract were dissolved in 2 liter of distilled water to get extract solution (1ml equivalent to 100 mg of purslane).

3. Experimental design

The initial and final body weight of rats were determined. Rats were divided into five groups (n=10). **1st group (control):** Rats were fed on normal chow diet (5% diet derived fat, 18% proteins, and 77% carbohydrates) for 18 weeks **(Svegliati-Baroni et al., 2006)**. **2nd group (HFD):** Rats were fed on high fat diet (58% fat, 18% protein, and 24% carbohydrates) **(Svegliati-Baroni et al., 2006)** for 18 weeks. **3rd group (HFD+Rest -4):** Rats were fed on high fat diet for 18 weeks then followed by diet restriction by feeding on normal chow diet for four weeks. **4th group (HFD+Rest -8):** Rats were fed on high fat diet for 18 weeks then followed by diet restriction by feeding on normal chow diet for eight weeks. **5th group (HFD+ purs. ext.):** Rats were fed on high fat diet and gavaged every day with purslane extract (1 g/ kg b.w.) **(El-Newary, 2012 and El-Newary, 2016)** for 18 weeks.

4. Sample Preparation:

Blood samples (6 ml/rat) were obtained from sinus orbitus vein of each rat **(Yang *et al.*, 2006)**, at the end of the experimental period. 2 ml of the blood was collected in a plastic centrifuge tube containing sodium citrate solution it was centrifuged immediately at 5000 rpm for 10 min. The supernatant plasma was immediately separated and stored at -20°C for determination of fibrinogen, coagulation factors (V/ VII/ X and von willebrand factor (vWF)] percentage, D-dimer and PAI-1 levels, prothrombin time (PT) and activated partial thromboplastin time (aPTT). Repeated freezing was avoided **(Nishizawa et al., 2002)**. 1.5 ml of the blood, each 0.5 ml was placed in a separate test tube, for calculation of whole blood clotting time (CT) **(Quick, 1966)**. The remaining amount (2.5 ml) of the blood was collected in clean plastic centrifuge tubes and allowed to clot. Serum was separated by centrifugation of blood at 3000 rpm for 15 minutes and stored at -20 C **(Nishizawa *et al,* 2002*)*** for determination of glucose, insulin, lipid profile, and leptin levels.

5. Biochemical assay:

- Measurement of serum glucose TC, TG, HDL-c levels according to **Tietz, (1995)**.
- Measurement of serum LDL-c and VLDL-c levels according to **Friedwald et al. (1972)**.
- Measurement of serum insulin levels according to **Temple *et al.*, (1992)**.
- The homeostasis model of assessment of insulin resistance (HOMA-IR) was calculated according to the following formula: [HOMA-IR = insulin (µU/mL) x glucose (mg/dl) /405] as an index of IR **(Matthews et al., 1985)**.
- Measurement of Leptin, plasma D-dimmers, PAI-1and vWF levels according to **Engvall & Perlmann, (1971)**.
- Determination of CT according to **Quick, (1966)**.
- Determination of PT and aPTT according to **Arkin, (1996)**.
- Measurement of plasma fibrinogen levels according to **Cooper and Douglas, (1991)**.
- Determination of plasma coagulation factors V, VII and X activities according to **Mannucci, *et al.* (2004)**.

6. Statistical Analysis

Data were presented as mean ± SE. The statistical analysis is done by using SPSS program (version 14) (SPSS Inc. Chicago, IL, USA). The obtained data were subjected to one way analysis of variance (ANOVA) to compare the treated groups together **Kirkwood (1989)**. All statements of significance were based on propability of $P<0.05$.

III. Results

Body weight and metabolic parameters (TC, TG, LDL-c, VLDL-c, glucose, insulin, HOMA-IR) and leptin levels illustrated in table (1) showed a significant ($P<0.05$) increase except HDL- c was decreased in all groups that were fed on HFD as compared with control group. Even though, caloric restriction (4 or 8 weeks) or purslane extract mitigate, to some extent, the disturbance effect of obesity on metabolic parameters, but most of them still significant as compared with control group.

All haemostatic parameters (fibrinogen, F.V, F.VII, F. X, D-dimmers, vWF, PAI-1) illustrated in table (2) showed a significant ($P<0.05$) increase except CT, PT& aPTT were decreased in all rat groups that were fed on HFD. Caloric restriction (4 or 8 weeks) or purslane extract alleviate the effects of induced obesity on haemostatic parameters but they still significant as compared with control group.

Table 1: Anthropometric measures (Mean±SE) and the metabolic parameters (Mean±SE) of the studied groups:

Parameters / Groups	Control (n=10)	HFD (n=10)	HFD + Rest -4 (n=10)	HFD + Rest -8 (n=10)	HFD + purs. ext. (n=10)
Initial BW (g)	164.40 ± 1.89	168.70 ± 2.72	164.00 ± 1.20	164.00 ± 1.20	167.50 ± 0.83
Final BW (g)	271.00 ± 6.48	416.30 ± 9.72 $^{a+}$	349.60 ± 5.79 $^{a+,b+}$	313.40 ± 6.27 $^{a+,b+}$	336.40 ± 5.13 $^{a+,b+}$
TC(mg/dl)	75.80 ± 1.94	189.60 ± 3.62 $^{a+}$	122.60 ± 2.91 $^{a+,b+}$	86.20 ± 2.44 $^{a+,b+}$	97.50 ± 3.05 $^{a+,b+}$
TG(mg/dl)	46.9± 1.95	150.00 ± 3.76 $^{a+}$	106.60 ± 3.62 $^{a+,b+}$	60.30 ± 2.90 $^{a+,b+}$	81.20 ± 1.30 $^{a+,b+}$
HDL-c (mg/dl)	42.50± 0.62	23.00 ± 2.83 $^{a+}$	30.9 ± 4.98 $^{a+,b+}$	39.2 ± 3.61 $^{a-,b+}$	35.50 ± 0.50 $^{a+,b+}$
LDL-c (mg/dl)	22.58 ± 1.51	136.60 ± 9.15 $^{a+}$	70.38 ± 10.60 $^{a+,b+}$	34.94 ± 5.21 $^{a+,b+}$	45.76 ± 11.00 $^{a+,b+}$
VLDL -c(mg/dl)	9.38 ± 0.39	30.00 ± 0.75 $^{a+}$	21.32 ± 0.72 $^{a+,b+}$	12.06 ± 0.58 $^{a+,b+}$	16.24 ± 0.26 $^{a+,b+}$
Glucose(mg/dl)	83.4 ± 2.47	237.7 ± 5.39 $^{a+}$	169.5 ± 5.37 $^{a+,b+}$	131.1 ± 3.57 $^{a+,b+}$	116.3 ± 3.08 $^{a+,b+}$
Insulin(uIU)/ml	19.44 ± 0.76	44.20 ± 1.28 $^{a+}$	36.5 ± 0.64 $^{a+,b+}$	28.79 ± 1.38 $^{a+,b+}$	23.95 ± 1.06 $^{a+,b+}$
HOMA-IR	4.02 ± 0.23	26.39 ± 1.41 $^{a+}$	15.35 ± 0.75 $^{a+,b+}$	9.43 ± 0.69 $^{a+,b+}$	6.94 ± 0.49 $^{a+,b+}$

Leptin	2.77 ± 0.13	10.51 ± 0.60 a+	6.42 ± 0.11 a+,b+	3.85 ± 0.17 a+,b+	4.59 ± 0.17 a+,b+

a+: Significant when compared with the control value (P 0.05).

b+: Significant when compared with HFD-group (P 0.05).

Table 2: The haemostatic parameters (Mean±SE) of the studied groups:

Parameters / Groups	Control (n=10)	HFD (n=10)	HFD + Rest -4 (n=10)	HFD + Rest -8 (n=10)	HFD + purs. ext. (n=10)
CT (sec)	120 ± 3.68	68.4 ± 3.06 a+	80.5 ± 1.97 a+,b+	109.4 ± 2.19 a-,b+	94.0 ± 2.24 a+,b+
PT (sec)	17.09 ± 0.33	9.36 ± 0.15 a+	14.35 ± 0.23 a+,b+	16.20 ± 0.22 a-,b+	15.94 ± 0.47 a-,b+
aPTT (sec)	25.44 ± 0.90	19.04 ± 0.28 a+	21.70 ± 0.33 a+,b+	23.88 ± 0.34 a-,b+	22.38 ± 0.27 a+,b+
Fibrinogen(mg/dl)	211.10 ± 7.95	360.80 ± 18.16 a+	295.10 ± 5.48 a+,b+	240.20 ± 4.59 a+,b+	251.10 ± 5.98 a+,b+
Factor V (%)	103.80 ± 2.70	127.70 ± 1.27 a+	121.00 ± 1.64 a+,b-	111.80 ± 3.24 a-,b+	115.50 ± 1.94 a+,b-
Factor VII (%)	105.10 ± 5.05	141.30 ± 3.31 a+	130.10 ± 3.73 a+,b-	117.50 ± 3.72 a+,b+	122.20 ± 3.07 a+,b+
Factor X (%)	107.50 ± 4.17	139.70 ± 4.36 a+	128.80 ± 2.34 a+,b-	119.20 ± 2.24 a+,b+	123.20 ± 3.10 a+,b+
D-Dimer (mg/dl)	66.22 ± 3.01	162.59 ± 15.31 a+	118.44 ± 3.51 a+,b+	93.20 ± 3.12 a+,b+	110.09 ± 3.55 a+,b+
vWF (%)	94.70 ± 2.36	137.10 ± 4.28 a+	119.20 ± 2.24 a+,b+	104.70 ± 2.87 a+,b+	111.10 ±3.18 a+,b+
PAI-1(ng/ml)	3.92 ± 0.27	16.82 ± 1.27 a+	12.72 ± 0.70 a+,b+	6.94 ± 0.52 a+,b+	8.75 ± 0.66 a+,b+

a+: Significant when compared with the control value (P 0.05).

a- : Non significant when compared with the control value (P 0.05).

b+: Significant when compared with HFD-group (P 0.05).

b- : Non significant when compared with HFD-group (P 0.05).

IV. Discussion

Rats that were fed with HFD for 18 weeks showed significant increase in b.w., lipid profile parameters (Cholesterol, triglyceride, LDL -c and VLDL-c), metabolic markers [glucose, insulin, HOMA- IR], levels of leptin, levels of plasma coagulation factors (fibrinogen, F.V, F.VII, F.X) and the levels of plasma fibrinolysis markers (D-dimer, VWF & PAI-1), but showed significant decrease in the levels of HDL and some blood coagulation markers (CT, PT & aPTT), as compared with control group.

It has been well-known that obesity was positively correlated with key metabolic syndrome markers such as elevated levels of b.w., triglycerides, total cholesterol, LDL-c, VLDL-c, plasma glucose, serum insulin and HOMA-IR, while, reduction of plasma HDL cholesterol is the most common lipid disturbance in obese state and is due in part to hypertriglyceridaemia **(Morgan & Edrees 2013 and Baltacı *et al*., 2016).** All these biomarkers induce risk of metabolic disturbance and promote oxidative stress state **(Lijnen *et al*., 2012)** as a result of increasing production of superoxide anions due to the high plasma levels of triglycerides and enhanced lipid peroxidation **(Giordano *et al*., 2011)**. Oxidative stress has been prescribed as the main mechanism responsible for metabolic disturbance while hypercholesterolemia under oxidative stress could trigger the progression of abnormal lipid metabolism. The generation of reactive oxygen species (ROS) including superoxide anions, hydrogen peroxide and hydroxyl radicals would react with unsaturated fatty acid chain in membrane lipids and cause lipid peroxidation which promotes health problems and metabolic disturbance **(Kai *et al*., 2015).**

These findings indicate a link between lipid profile and insulin sensitivity, since systemic excess of free fatty acids impairs the ability of insulin to stimulate glucose metabolism, contributing to whole body insulin resistance **(Schaalan *et al*., 2009).** So, one of the most important mechanisms by which obesity leads to the development of metabolic disorders is the promotion of insulin resistance in skeletal muscles, adipose tissue and liver. The adipose tissue excess, particularly visceral fat, is associated with a continuous production of mediators that impair insulin action in skeletal muscles, like free fatty acids. Additionally, in insulin resistance and obesity, the dyslipidaemia is characterized by a different composition and distribution of LDL-c, resulting in an increased concentration of the more atherogenic small dense LDL-c. Small dense LDL-c particles can move through endothelial fenestrations, entering the subendothelial space where inflammation and transformation into plaque can occur **(Tesauro *et al*., 2011)**. Furthermore, the oxidized low-density lipoprotein is mostly taken up by macrophage scavenger receptors, rather than the normal LDL-c receptor pathway thus inducing metabolic disturbances **(Houstis *et al*., 2006).**

obesity is frequently accompanied by a state of hyperinsulinemia and impaired glucose tolerance, elevated triglycerides, decreased HDL-c levels and hypertension that are all described under the heading of the metabolic syndrome **(Galli-Tsinopoulou *et al*., 2011 and Orenes-Piñero *et al*., 2015)**.

PAI-1 is typically increased in the obesity / IR state and plays an important role in the genesis of vascular abnormalities**.** Also obesity and IR are frequently associated with altered coagulation/ fibrinolysis. In combination, all of the above abnormalities create a state of constant and progressive damage to the vascular wall, manifested by a low- grade progressive inflammatory process. Endothelial dysfunction is defined as inadequate endothelial mediated vasodilation and is characterized by loss of balance between vasoconstrictors and vasodilators, increased oxidative stress, and elevated expression of proinflammatory and prothrombotic factors. Endothelial dysfunction is a fundamental initial step in the development and progression of atherosclerosis and has been considered an important event in the development of microvascular complication **(Caballero, 2003, Picchi *et al*., 2006 and Park *et al*., 2016)**.

Leptin is not only a marker of weight gain but also seems to have a relationship with developing a systemic state of low- grade inflammation and metabolic disturbance. Hyperleptinemia showed increased plasma levels of inflammatory cytokines, hyperglycemia, hyperinsulinemia, hyperlipidemia, liver steatosis and insulin resistance **(Leon-Cabrera *et al*., 2013)**. And it is directly correlates with higher plasma levels of prothrombotic factors such as vWF, F.VII and PAI-1, which lead to a higher risk of thrombosis and atherosclerosis **Tesauro *et al*., (2011)**. Leptin regulates feeding behavior through the central nervous system and there is a positive relation between leptin and the increased body fat accumulation leading to increased BMI and disturbance of lipid profile **(EL-Wakf *et al*., 2015 and Kim *et al*., 2015)**. With all of this, there is much evidence supporting the theory that leptin acts via the hypothalamus to suppress food intake and to increase energy expenditure by modulating glucose and fat metabolism, and enhancing thermogenesis. In addition, the leptin receptor is present in many tissues, including platelets. Leptin promotes human platelets aggregation by potentiating the normal platelet response to its agonists adenosine diphosphate and thrombin. This has been suggested as a mechanism for acute thrombotic events in obesity **(Darvall *et al*., 2007, and Anfossi *et al*., 2010).** Furthermore, leptin have multiple effects on the cells of the arterial wall and upregulates the expression of the PAI-1 and cholesterol efflux from macrophages **(Hongo *et al*.,**

2009). In addition, leptin has been reported to increase NO bioavailability in blood vessels via the activation of endothelial NO synthase and inducible NO synthase in the endothelial and smooth muscle cells respectively **(Wang & Nakayama, 2010).**

Fibrinogen, an acute phase reactant like C - reactive protein, rises in response to a high cytokine state. Thus, prothrombotic and proinflammatory states may be metabolically interconnected **(Taura *et al*., 2014).** The obese patients continually have metabolic disturbances and high plasma concentration of fibrinogen, vWF, VII, VIII, and X factors, in addition, the fibrinolytic parameters PAI-1, tissue-type plasminogen activator (t-PA) antigen and vWF are strongly correlated to the components of the metabolic syndrome (especially abdominal obesity, IR, and hypertriglyceridemia) and that the improvement of IR may improve the concentration of the fibrinolytic parameters **(Cornier *et al*, 2008 and Franchini *et al*., 2010)**. The obesity is associated with an early endothelial damage, confirmed by elevated level of vWF-antigen (a marker of endothelial activation). Evaluation of plasma levels of haemostatic markers of endothelial dysfunction as vWF- antigen and PAI-1 antigen, preceded new cases of type 2 diabetes and other major diabetes risk factor such as obesity, IR and inflammation **(Mauras *et al*., 2010).** In addition, high levels of D- dimmer which is a product of plasmin digestion of cross- linked fibrin were positively associated with BMI and vWF in obese patients. There is also a correlation between cholesterol and D- dimmer. So, probably the excess of adiposity may cause an imbalance in the haemostatic system, wherein more fibrin is produced and deposited **(Giordano *et al*., 2011)**.

Moreover, a significant increase in PAI-1, vWF, D- dimmer and fibrinogen level, while, a significant decrease in whole blood clotting time, bleeding time, prothrombin time, activated partial thromboplastin time indicate hypercoagulable state **(Hossen *et al*., 2010 and Asalah *et al*., 2014)**.

From all the previous reports, we can conclude, one of potential mechanism by which excess adipose tissue contributes directly to the prothrombotic state is (i) impairing platelet function via low- grade inflammation and increase in circulating leptin, (ii) impairing fibrinolysis by production of PAI-1 and possibly thrombin – activator fibrinolysis inhibitor, (iii) impairing coagulation by release of tissue factor, and (iv) affecting hepatic synthesis of the coagulation factors fibrinogen, factor V, factor VII, factor VIII and tissue factor, by releasing free fatty acids and proinflammatory cytokines into the portal circulation and by inducing hepatic IR **(Denison *et al*., 2010 and Kaye *et al*., 2012).** Also, a positive association between the measures of obesity, and plasma fibrinogen, increases in the activities of fibrinogen, clotting factors IX, X, XI and PAI-1 was observed in obese. These simultaneous activities of intrinsic coagulation factors and impaired fibrinolysis could be considered as a predisposing factor for thrombosis **(Morgan & Edrees, 2013, Phelan & Kerins, 2014 and Kim *et al*., 2016)**.

The present data revealed that HFD-rats for 18 weeks then followed by diet restriction (4 or 8 weeks) and that were gavaged daily with 1g/ kg b.w. of purslane extract during feeding with HFD showed significant decrease in the levels of b.w., lipid profile parameters (TC, TG, LDL-c,VLDL-c), metabolic markers (glucose, insulin, insulin resistance), leptin and the levels of plasma endothelial dysfunction markers (fibrinogen, F.V, F.VII, F.X, D-dimmer, vWF, PAI-1) but showed significant increase in the levels of HDL and some blood coagulation markers (CT, PT, aPTT) as compared with HFD-group. These results indicate that diet restriction specially for 8 weeks and purslane extract treatment cause weight loss, to an extent, that b.w. may reach about normal values. This weight loss was accompanied with improvement of lipid profile and plasma metabolic parameters and reduced coagulation disorder. This may be due to reduction of lipid peroxidation and ROS generation, thereby mitigate oxidative stress state. So, the body weight loss and body fat reduction correct metabolic disorder and improve coagulation and inflammation profile and potentially reducing the thrombotic risk. weight loss and a decrease in serum LDL-c and triglyceride concentrations, and an increase in serum HDL where HDL particles possess potent antioxidative activity and therefore the oxidative stress is alleviated **(Darvall *et al*., 2007)**.

Weight loss is accompanied by an improvement in IR and reduction in plasma triglycerides and glucose levels. These metabolic changes may account for the improvement of the hematological profile and fibrinolytic activity and a decrease in PAI-1 levels which in turn results in a reduction of other fibrinolysis markers (D-dimmer, vWF and fibrinogen) which is the cause of thromboembolism in severely obese individuals **(Pardina *et al*., 2012 and Phelan**

& Kerins 2014). So, restoring normal body weight is an appropriate strategy for reducing endothelial dysfunction, inflammatory mediators and thrombotic risk **(Kim *et al*., 2015 and Korybalska *et al*., 2016)**.

The presence of phytochemicals including glycosides, flavonoids, alkaloids, terpenoids, phenolic and other antioxidant compounds in purslane extract could possess serum lipid lowering properties that help in preventing or slowing the progress of obesity associated diseases **(Kamal Uddin *et al*., 2014 and Sadeghi *et al*., 2016)**.

Dietary fibers content of purslane, especially viscous soluble polysaccharides, are well known for their lowering effect of total cholesterol and LDL-c thus attenuating or preventing hypertriglyceridemia. Also, viscous soluble fibers hinder digestion and absorption of dietary fats, resulting in lower cholesterol delivery to the liver by chylomicrons remnants, with a concomitant up regulation of LDL-c receptor and decreased lipoprotein secretion to maintain cholesterol homeostasis in the liver. So, the lower serum level of cholesterol and triglycerides leads to the reduced risk of cardiovascular disease associated with dietary fiber intake. **(Anderson *et al*., 2000 and Jalili *et al*., 2001).**

Polyphenol- rich foods – as purslane – has been associated with decreased intestinal absorption of TG by inhibition of pancreatic lipase and increased lipoprotein lipase activity. This could result in modified LDL-c levels, which in turn would lead to decreased LDL-c concentration **(Ikeda *et al*., 2005 and Bursill *et al*., 2007).**

Moreover, alcoholic extract of purslane has an anti-coagulant effects as evidenced by significant increase in CT, PT and aPTT, while significant decrease in coagulation factors V, VII and X levels. These hypocoagulant effects of purslane may be attributed to its content of omega-3 fatty acids and antioxidant **(Essa *et al*. 2007)**. The high omega-3 fatty acids level in Purslane may be responsible for the triglyceride levels reduction. Purslane was effective in lowering plasma LDL -c and increasing HDL-c in hypercholesterolemic subjects. The high pectin level in Purslane may be responsible for its hypocholesterolemic effect **(Tabatabaie and Shahbaz Boroujeni, 2015**).

The levels of vitamin K dependant coagulation factors VII, X and prothrombin and vitamin K-independant factor V were found to be reduced by high administration of omega-3 fatty acids, which resulted in hypocoagulant effect. One of the widely accepted mechanisms by which vitamin K dependant coagulation factors are reduced after omega-3 fatty acids intake was as follow, vitamin K is a lipophilic molecule which needs to be transported through the blood in a lipid bound way. Feeding with omega-3 fatty acids leads to strong reduction in lipoprotein-bound cholesterol and triglycerides that will limit transport of vitamin K and thereby synthesis of vitamin K dependant coagulation factors in the liver **(Nieuwenhuys *et al*., 2001)**.

Also, oral administration of purslane extract to diabetic rats showed a significant reduction in the level of glucose associated with significant improvement in the level of insulin. The conceivable mechanism of lowering blood glucose level by purslane extract is the increase of insulin secretion either by direct stimulation of insulin release from pancreatic -cells or preservation of residual -cell functions. Other mechanisms like increasing the responsiveness to insulin, - receptor agonists, or direct insulin-like activity cannot also be ruled out **(El-Sherbiny *et al*., 2005)**.

Several mechanisms might explain the antioxidant effects of purslane. Purslane can increases insulin secretion by closing the gate of ATP-sensitive K^+ channel leading to membrane depolarization, and opening of voltage gated L-type Ca^{2+} channel allowing Ca^{2+} entry as the first key step in stimulation of insulin secretion by exocytosis. Improved homeostasis of glucose in diabetic patients might explain the beneficial effects of purslane on biomarkers of oxidative stress. Purslane has been shown to elevate antioxidant enzymes through which it might lead to reduced oxidative stress **(Zakizadeh *et al*., 2015)**.

Additionally, purslane extract contains linoleic acid which plays an important role in reduction of total cholesterol level **(Kai *et al*., 2015).** Moreover, the bioactive compounds such as phytosterols and ascorbic acid which are present in purslane extract play their role in lowering serum cholesterol level. Phytosterol (β-sitosterol) could

increase cholesterol excretion from the body, besides it help in inhibition of cholesterol absorption, lower the triglycerides and increase HDL **(Khandelwal *et al*., 2013).**

Moreover purslane extract is a rich source of antioxidant vitamins. Alpha-tocopherol is the biologically active form of vitamin E. It is a chain-breaking antioxidant that prevent the propagation of free radicals activities. It has chromanol ring with hydroxyl group which can donate hydrogen atom to reduce free radicals and hydrophobic side chain which allow for penetration into the biological membrane. It protects the poly unsaturated fatty acids within the membrane phospholipids, plasma lipoprotein and low density lipoproteins against oxidation **(Rosenau *et al*., 2007)**. The mechanism by which vitamin E exert hypocoagulant effect is that alpha tocopherol can decrease the gamma carboxylation and functionality of prothrombin, a vitamin K-dependant protein among adults not receiving oral anticoagulant therapy. It also inhibit platelet aggregation and adhesion **(Booth *et al*., 2004)**. Endothelium in the vascular system is an important modulator of vasomotor tone and coagulation. Vitamin E inhibits the activation of endothelial cells stimulated by high levels of low density lipoprotein cholesterol and proinflammatory cytokines. This inhibition is associated with supression of chemokines **(Meydani, 2004)**.

Beta carotene and vitamin C in crude purslane extract has a weak haemorrhagic effects which may be attributed to the decreased effect of vitamin K dependant coagulation factors and also prothrombin time was prolonged **(Liu *et al*., 2000 and Simopoulos *et al*., 2005)**. Also crude purslane extract contain L- arginine, which can result in a two fold increase in prothrombin time so it inhibits the activation of hemostasis and can be used as a hemostatic stabilizer **(Stief *et al*., 2001).**

Furthermore, purslane extract is a good source of coenzyme Q10 (Co Q10), which is a non specific antioxidant and is classified as a fat soluble quinone with chemical structure similar to that of vitamin K. The primary clinical applications of CoQ10 are in prevention and treatment of cardiovascular diseases and diabetes, **(Bonakdar and Guarneri, 2005)**. In addition, CoQ10 has been demonstrated to have potent antioxidant effects against lipid peroxidation and may offer protection against atherosclerosis by preventing lipid peroxide formation and oxidation of low-density lipoprotein cholesterol **(Ross, 2007).**

Purslane extract also contains catecholamines**.** These catecholamines are able to down-regulate PAI-1expression and secretion in human adipocytes via beta-adrenergic receptors **(Gottschling-Zeller *et al*., 1999 and Obied *et al*., 2003)**.

V. Conclusion

HFD-induced obesity in rats is associated with hypercoagulability and impairs fibrinolytic activity, which lead to a higher risk of thrombosis. Diet restriction plays a pivotal role in regulating obesity and thrombosis potential. As well as, purslane extract have ameliorative effect on obese metabolic alteration and markers of blood coagulation and endothelial dysfunction of obesity, so, purslane extract may match diet restriction effect but it is more safer. More studies must be done to reveal other purslane benefits.

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