Hypolipidemic and cardioprotective effects of *Taraxacum officinal* aqueous extract in obese rats

Toumi Ikram, Medila Ifriqya and Lifa Saoussane*

Abstract

Obesity is a chronic disease responsible for comorbidity and excess mortality, and is considered an independent risk factor for cardiovascular pathology development. Most cardiovascular disease can be prevented by tackling behavioural risk factors, such as a sedentary lifestyle, unhealthy diet, and obesity. *Taraxacum officinal* is a perennial plant belonging to the *Asteraceae* family, commonly used for these medicinal characteristics. It has diuretic, anti-tumour, antioxidant, anti-inflammatory, hepatoprotective and immunostimulant properties. The aim of this study was to evaluate the lipid-lowering and cardioprotective effect of *Taraxacum officinal* aqueous extract in Wistar rats on hyper-fatty diets. A total of 24 rats weighing 200 ± 6.8 g were divided into three lots: healthy control (HC) receiving a standard diet, obese control (OC) receiving a cafeteria diet without treatment and the third load (TL) receiving a cafeteria diet and treated for 20 days with 200 mg/kg *Taraxacum officinal* aqueous extract. The results showed that the cafeteria diet induced obesity in rats compared to the control group, characterized by hyperglycaemia (148.75 mg/dL), hypertriglyceridemia (59 mg/dL) and hypercholesterolemia (160.67 mg/dL) with an increase in total lipids (0.39 g/g of tissue) associated with a state of oxidative stress in the cardiac tissue. Oral administration of the aqueous extract of *Taraxacum officinal* improved the lipid profile in serum and tissue. The findings showed a drop in blood sugar (1.02 mg/dL), total cholesterol (135 mg/dL), LDL cholesterol, (67 mg/dL), triglycerides (36 mg/dL), total lipids (1.37g/g of tissue), and lipid peroxidation MDA (0.25 ± 0.02 µmoL/g protein), and an increase in the level of GSH (0.51 nM /mg protein) in treated rats compared to the controls. In conclusion, the results obtained showed the effectiveness of the aqueous extract of *Taraxacum officinal* against dyslipidemia, obesity, and hyperglycaemia. The plant was shown to have both cardioprotective and antioxidant effect.

Key words: *Cardiac disease; Dyslipidemia; Obesity; Oxidative stress; Risk factor; Taraxacum officinal*

Toumi IKRAM*, DVM, Senior Professor, (Corresponding author, e-mail: toumi-ikram@univ-eloued.dz), Medila IFRIQYA, DVM, Senior Professor, Department of Cellular and Molecular Biology, Life and Natural Sciences Faculty, University of Hamma Lakhdar El Oued, 39000, Algeria, Biology, Environment and Health Laboratory, University of Hamma Lakhdar El Oued, 39000, Algeria; Lifa SAOUSSANE, Assistant Master, Biology, Environment and Health Laboratory, University of Hamma Lakhdar El Oued, 39000, Algeria

Introduction

The key risk factors for cardiac disease and stroke are unhealthy diet, lack of physical activity, smoking and excessive alcohol intake. The consequences of lifestyle risk factors can affect people with hypertension, hyperglycaemia, hyperlipidaemia, excessive body weight and obesity. Obesity is the state of an organism with excess adiposities or excess fat mass resulting from a positive energy balance, in proportions that may have negative health effects. Visceral and massive obesity is a well-established risk factor for high blood pressure (hypertension), coronary heart disease and excess cardiovascular mortality (Corcos, 2012). The risk of coronary artery disease in obese and overweight people is partially explained by the frequent coexistence of other cardiovascular risk factors. Studies have also shown that there is a longitudinal linear relationship between obesity and coronary artery disease (Jousilahti et al., 1996).

Obese subjects are often distinguished by a dyslipidemic state in which plasma triglycerides are increased, HDL-C concentrations are lowered, and LDL apolipoprotein (Apo B-100) concentrations are increased. Hence central fat distribution plays a significant role in lipid abnormalities (Despres and Lemieux, 2006). The main risk of these pathologies is the appearance of cardiovascular disease through the formation of atheroma plaques that gradually block the arteries, causing atherosclerosis. Hypertension, coronary insufficiency, stroke, venous thrombosis and pulmonary embolism are major cardiovascular diseases associated with obesity. Another disorder is heart failure, which results from fatigue of the heart caused by excess body weight and the associated complications (Must et al., 1999; Hensrud and Klein, 2006). Given the side effects of surgery and the harmful effects of synthetic drugs on weight loss, natural products are a better option in the treatment of excessive body weight, obesity and other medical disorders due to their efficacy (James, 2017).

Taraxacum officinal is a perennial plant belonging to the *Asteraceae* family, commonly used for its medicinal characteristics. It has diuretic, anti-tumour, antioxidant, anti-inflammatory, and hepatoprotective activity (Baba et al., 1981; Kisiel and Barszcz, 2000; Hu and Kitts, 2003; Jeon et al., 2008). The aim of this study was to evaluate the lipid-lowering and cardioprotective effect of *Taraxacum officinal* aqueous extract in obese Wistar rats.

Materials and methods

Aqueous extract preparation

A total of 10 g powdered leaves dissolved in 150 ml distilled water was heated to reflux for 2 hours. After cold filtration; this filtrate was then evaporated to dryness reduced to 65°C using a rotating evaporator.

Animals and Obesity induction

The experiment included 24 Wistar rats weighing 200±6.8 g from the Pasteur Institute, Algeria. Rats were acclimatized for two weeks under the conditions of the Molecular and Cell Biology Department, El Oued University, Algeria: air temperature of 22±2 °C, 50% humidity and a normal photoperiod (12 h light/12 h darkness).

To induce obesity, rats were given either a standard diet (control) or the cafeteria diet for a period of one month. The cafeteria diet is made up of 50% standard diet and 50% sausage mixture, dry cookies, cheese, chips, peanut, and chocolate in the ratio 2:2:1:1:1 (Darimont et al., 2004). Obesity perception in rats was confirmed by monitoring body

weight gain and the quantity of food consumed during one month.

Treatment of animals

After one month, non-obese rats served as the healthy control lot (HC) receiving a standard diet with tap water. Obese rats were divided into two groups: obese control (OC) receiving a cafeteria diet without treatment, and the third load (TL) receiving a cafeteria diet and treated for 20 days with 200 mg/kg *Taraxacum officinale* aqueous extract.

Sacrifice, blood and organs samples

Rats were anesthetized with chloroform (94%) and sacrificed after 12 hours of fasting. Blood samples were collected in EDTA and dry tubes. After centrifugation at 3000 rpm for 15 minutes, serum and plasma were collected and stored for lipid parameter determination.

The liver, heart, adipose tissue, and kidneys were carefully removed after dissection, rinsed with saline solution, and weighed. Organ homogenates were used to evaluate oxidative stress parameters and to measure tissue lipids.

Dosage of serum and tissue lipid parameters

Triglycerides (TG), total cholesterol, HDL-cholesterol and glutamate-oxalo-acétate-transaminase (GOT) were determined using the colorimetric method by a type autoanalyzer (BIOLIS24j) with the appropriate reagent package for each parameter. LDL-cholesterol was determined using the direct calculation method according to the formula of Friedwald et al. (1972): LDL-C = Total cholesterol - [HDL-C + TG / 5].

For the quantification of total lipids at the tissue level, we used cold extraction with a mixture of polar/apolar solvents (chloroform/methanol) according to the Folch et al. (1957) method.

Oxidative stress parameters

To prepare homogeneous organs, 1 gram of tissue (heart, liver, kidney and adipose tissue) from each rat was used from the various study groups. After grinding and homogenizing the tissues in TBS (Tris 50 mM, NaCl 150 m M, pH 7.4), samples were centrifuged at 3000 r/min for 15 min. The supernatant was recuperated to perform the oxidative stress parameter assay.

The thiobarbituric acid method (TBA; Yagi, 1976) is used to determine malondialdehyde (MDA) which reacts with TBA to give pink absorbing chromophores at 532 nm. Glutathione (GSH) was determined by a SHIMATZU type spectrophotometer using the colorimetric method (Weckbecker and Cory, 1988); the measurement of optical density was the result of the formation of 2-nitro-5 mercocapturic acid from the reduction of dithio-bis2 nitrobenzoic acid. Absorbance was set at 412 nm.

Statistical analysis

The results are presented as mean ± standard deviation. The comparison of means was carried out by the Student t-test using MINITAB and EXCEL software. Differences were considered significant at $P<0.05$.

Results

Food consumption, weight gain and total fat content in tissue

The results obtained are presented in Table 1.

During treatment, there was no difference in the amount of food consumed by rats receiving the cafeteria diet compared to the control lot. Treatment with *Taraxacum officinal* aqueous extract significantly decreased food intake in obese rats compared to the control (HC). The results showed that the cafeteria diet induced an increase in weight gain and

Table 1. Food consumption and weight gain in control rats, obese controls and treated rats (mean ± standard error).

Parameter	HC	OC	TL
Food consumption (g/d/rat)	28.375±0.95	29.483±0.54^{NS}	24.583±0.73^{***,c}
Weight gain (g/d)	0.325±0.01	0.677±0.13*	0.021±0.1^{*,c}
Fat total content in tissue (g)	0.152±0.01	0.49±0.02***	0.143±0.05^{NS,c}

Comparison with control group (C): *** P<0.001, with obese control group (OC):
^{a}P<0.05; ^{c}P< 0.001, n=8 rats

Table 2. Plasma concentration of glucose, triglycerides (TG), cholesterol (CL), HDL and LDL in the control and treated groups (mean ± standard error).

Parameter	Control group	Obese control group	Treated group
Glycaemia (mg/dL)	97.75±4.37	148.75±4.35***	102.33±5.36^{NS,a}
Triglyceride (mg/dL)	43±7.94	59±3.51*	36.67±3.33^{NS,a}
Cholesterol (mg/dL)	142±7.09	160.67±1.86**	135.33±4.48^{NS,a}
HDL (mg/dL)	60.66±0.33	52.3±3.68^{NS}	61 ±4.58^{NS}
LDL (mg/dL)	78.07±5.64	96.57±2.35^{NS}	67±0.577^{***,c}

Comparison with control group (C): ***P<0.001, **P<0.01, *P<0.05 with obese control group (OC):
^{a}P<0.05; ^{c}P< 0.001, n=8 rats

tissue lipid content in obese control rats relative to the control group. In the Wistar rat, consuming a hyperlipidic and high-calorie diet increases food intake, body weight and induces lipid accumulation in adipose tissue (Milagro et al., 2006; Bouanane et al., 2010; Benkalfat et al., 2011). The accumulation of adipose tissue and its lipid enrichment is a feature of the cafeteria diet induced by obesity (Caluwaerts et al., 2007). Lipotoxicity results from an ectopic accumulation of lipids in the liver, and the muscles and heart are involved in the insulin resistance of these different tissues (Despres and Lemieux, 2006).

However, the administration of *Taraxacum officinal* aqueous extract causes a considerable reduction in weight gain and in tissue lipid content in the treated group (TL) compared to the obese control group (OC). This decrease in weight may be due to several mechanisms. According to Kajimura and Saito, (2014), most natural anti-obesity products (medicinal plants) regulate body weight through an increase in compulsory energy expenditure by transforming energy from food into heat.

Blood glucose and lipid content

The results showed a significant increase in glycaemia, triglycerides, cholesterol and LDL-C levels, and a decrease in HDL-C levels in the OC group compared to the control group C. However, significant decreases (P<0.01) in glucose and serum lipids were observed in the treated group (TL) (Table 2).

Obesity caused by the cafeteria diet induced an increase in blood sugar. Studies have reported that rats with a diet high in fat develop insulin resistance and confirmed hyperglycaemia (Stubbs and Wickremesekera 2002; Bihan et al., 2007). The hyper lipid diet increases glucose production by reducing insulin suppression and increasing

gluconeogenesis (Martínez-Gonzalez et al., 1989), resulting in elevated plasma glucose levels. Studies of *Taraxacum officinal* extracts revealed that it can stimulate insulin release into the β cells of the pancreas, thus neutralizing the effects of hyperglycaemia (Hussain et al., 2004; Schütz et al., 2006).

The results indicate an observed hyperlipidaemia in OC rats, which can be explained by the high lipid content in the diet. Obesity is also distinguished by an expansion of the adipose tissue mass and an increase in the cholesterol and triglyceride storage capacity (Benkalfat et al., 2011), which raises the risk of cardiovascular disease. Kim et al. (2000) identified that triglyceride accumulation was associated with insulin resistance as a result of insulin signalling disruption in tissues.

The results also showed a significant increase in LDH cholesterol. The increased serum LDH activity is a precursor for necrosis of cardiac tissue (Ben Amor et al., 1999). The administration of aqueous extract of *Taraxacum officinale* decreased the serum level of LDH thus inducing a decrease in cardiac tissue necrosis. In rats, *Taraxacum officinal* leaf extract has been shown to reduce serum glucose, cholesterol and triglyceride levels, possibly due to the elevation of protein kinase activated by adenosine monophosphate (AMPK) in the liver, resulting in a significant decrease in the accumulation of lipids and an improvement in sensitivity to insulin (Davaatseren et al., 2013). Several studies have reported the presence of saponins in the *Taraxacum officinal* aqueous extract (Mir et al., 2013), the latter having anti-hyperlipidaemia and anti-hypercholesterolemia effect (Özlem and Giuseppe, 2007).

GOT levels and oxidative stress biomarkers in cardiac tissue

The results obtained show an increase in the serum activity of GOT in the obese control group compared to the control group ($P<0.01$; Figure 1). The increase in serum transaminase activity (GOT) in obese rats is considered to be a biomarker of hepatic dysfunction or cardiac damage (Fiacre et al., 2002; Dieusaert, 2005) caused by the hyper fat diet. However, GOT was reduced after the administration of *Taraxacum offilcinal* aqueous extract, and this effect could be interpreted as a protective effect of the cardiovascular system.

The results show a significant increase in the malondialdehyde (MDA) concentration (Table 3) in cardiac tissue

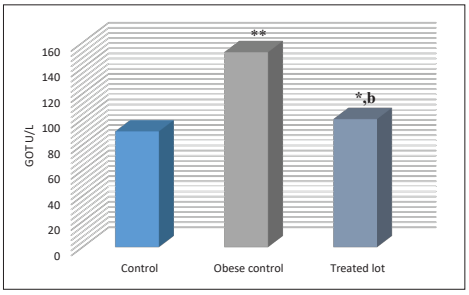

Figure 1. Activity of GOT transaminases in control groups and treated group.
Comparison with control group (C): ** $P<0.01$, with obese control group (OC): $^{b}P< 0.01$, n=8 rats

Table 3. Cardiac tissue concentrations of malondialdehyde (MDA) and reduced glutathione (GSH) in the control, obese control and treated group (mean ± standard error).

	Control	Obese control	Treated group
MDA (μmoL/g tissue)	0.322±0.05	0.522±0.058*	0.232±0.026*,b
GSH (nM/mg protein)	0.168±0.015	0.089±0.007**	0.367±0.053*,b

Comparison with control group (C): ** $P<0.01$, * $P<0.05$ with obese control group (OC): $^{b}P< 0.01$, n=8 rats

in obese controls compared with healthy controls, which favours apparent oxidative stress.

These results can be explained by lipid self-oxidation (Saka et al., 2011), likely induced by obesity. A high-fat diet also leads to mitochondrial dysfunction correlated with oxidative stress (Yuzefovych et al., 2013). Nevertheless, a significant decrease in MDA was observed in the *Taraxacum officinal* extract treated group, showing the antioxidant effect of the plant (Hagymasi et al., 2000; Choi et al., 2010; Gonzalez-Castejon et al., 2012).

The results of the statistical analysis show evidence a significant reduction of GSH in the cardiac tissue in obese rats. On the one hand, this reduction can be viewed as an increase in its use by liver cells, while on the other, by a reduction in the synthesis of GSH or increase of its degradation during oxidative stress (Loven et al., 1986).

GSH can be associated with many pathways in vascular physiology and pathology. Oxyradicals and peroxides are considered to have strong effects on platelets, endothelial cells and smooth vascular muscle cells (Hennig and Chow, 1988; Rubanyi, 1988). As a product of oxidative stress, endothelial dysfunction has been identified as a possible pathological vector in the formation of atherosclerosis, and in vascular sound alterations and permeability (Boissonneault et al., 1990).

However, a reduction in glutathione was observed in obese rats after administration of the aqueous extract of *Taraxacum officinal*. Ivanov, (2014) reported that *Taraxacum officinal* is a good source of biologically active compounds and has desirable antioxidant properties. Raising glutathione levels fights the oxidation of fatty acids in the bloodstream, including cholesterol, thereby delaying the process of plaque formation in the arteries, which is the underlying cause of most heart problems (Stamler and Slivka, 1996). Additionally, GSH plays an important role in leukotrien and prostaglandin formation and metabolism, which have a potential role in coronary artery constriction, causing ionotropic damage to cardiac muscle. The results also showed that the *Taraxacum officinal* aqueous extract improved the state of oxidative stress in rats, confirming that this plant could contribute to the protection of the heart against pathologies linked to the deleterious effects of reactive oxygen species.

Conclusions

Taraxacum officinal aqueous extract had a cholesterol-lowering, hypotriglyceridemic, hypoglycaemic and cardioprotective effect in Wistar rats.

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Hipolipidemijski i kardioprotektivni učinak vodenog ekstrakta *Taraxacum officinal* u pretilih štakora

Toumi IKRAM, dr. med. vet., redovita profesorica, dr. sc. Medila IFRIQYA, dr. med. vet., redovita profesorica, Zavod za staničnu i molekularnu biologiju, Prirodoslovni fakultet, Univerzitet u Hamma Lakhdar El Oued, 39000, Alžir, Biologija, Laboratorij za okoliš i zdravlje, Univerzitet u Hamma Lakhdar El Oued, 39000, Alžir; Lifa SAOUSSANE, viši asistent, Biologija, Laboratorij za okoliš i zdravlje, Univerzitet u Hamma Lakhdar El Oued, 39000, Alžir

Pretilost je kronična bolest koja je odgovorna za komorbiditet i prekomjernu smrtnost te se smatra neovisnim čimbenikom rizika za razvoj kardiovaskularne patologije. Većinu kardiovaskularnih bolesti moguće je spriječiti rješavanjem faktora rizika ponašanja; sjedilačkog načina života, nezdrave prehrane i pretilosti. *Taraxacum officinal* je višegodišnja biljka koja pripada obitelji *Asteraceae*, a obično se rabi zbog svojih medicinskih svojstava. Ima diuretičko, protutumorsko, antioksidativno, protuupalno, hepatoprotektivno i imunostimulacijsko djelovanje. Cilj je ove studije bio procijeniti učinak snižavanja lipida i kardioprotektivni učinak vodenog ekstrakta *Taraxacum officinal* u štakora soja wistar na vrlo masnoj prehrani. 24 štakora težine 200 ± 6,8 g podijeljeno je u 3 skupine: zdravu kontrolnu (HC) koja je primala standardnu prehranu, pretilu kontrolnu (OC) koja je primala „kantinsku" hranu bez terapije i treću opterećenu skupinu (TL) koja je primala „kantinsku dijetu" i terapiju tijekom 20 dana s 200 mg/kg vodenog ekstrakta *Taraxacum officinal*. Dobiveni rezultati su pokazali da je „kantinska dijeta" dovela do pretilosti štakora u usporedbi s kontrolnom skupinom okarakteriziranom hiperglikemijom (148,75 mg/dL), hipertrigliceridemijom (59 mg/dL) i hiperkolesterolemijom (160,67 mg/dL) uz povećanje ukupnih lipida (0,39 g/g tkiva) povezano sa stanjem oksidativnog stresa u srčanom tkivu. Oralna primjena vodenog ekstrakta *Taraxacum officinal* poboljšala je lipidni profil u krvi i tkivu. Nalazi su pokazali pad šećera u krvi (1,02 mg/dL), ukupnog kolesterola (135 mg/dL), LDL kolesterola, (67 mg/dL), triglicerida (36 mg/dL), ukupnih lipida (1,37 g/g tkiva) i peroksidacije lipida MDA (0,25 ± 0,02 μmoL/g proteina) te povećanje razine GSH (0,51 nM/mg proteina) u štakora koji su primali terapiju u usporedbi s kontrolnim skupinama. Zaključno, dobiveni rezultati dokazali su učinkovitost vodenog ekstrakta *Taraxacum officinal* protiv dislipidemije, pretilosti i hiperglikemije. Biljka ima kardioprotektivni i antioksidativni učinak.

Ključne riječi: *bolest srca, dislipidemija, pretilost, oksidativni stres, faktor rizika, Taraxacum officinal*