الجمهورية الجزائرية الديمقراطية الشعبية
People's Democratic Republic of Algeria

وزارة التعليم العالي و البحث العلمي
Ministry of Higher Education and Scientific Research

University of brothers Mentouri Constantine

جامعة الإخوة منتوري قسنطينة

Faculty of Natural Sciences and Life

كلية علوم الطبيعة و الحياة

قسم الكيمياء الحيويــة و البيولوجيــا الخلـــوية و الجزيـئـية
Department of Biochemistry /Molecular and Cellular Biology

Dissertation

To Get a Diploma of Master in (Fundamental Biochemistry)

Option: Proteomic Analysis and Health

Entitled :

Effect of Dietary Addition of 5% *Pistacia lentiscus* oil on Serum Lipids and Body Fat in Rats

Done by:

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Presented on June 30th 2015

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Academic year: 2014-2015

DEDICATION

I dedicate my dissertation work to my family and my friends.

A special feeling of gratitude to my loving parents, for their continual faithful support during my study.

Thanks and gratitude are also extended, to my brothers, Houssem eddine and Hamza, my sister, Meriem.

I also pay thankfulness to my best friends; boubakeur , Seif eddine, Chams eddine, Alla Eddine, Zakaria, Hammoudi ,Abdelsalam, Djamel Eddine,Charaf Eddine, Asma, Hadjer and Sarah .

Finally, I pray that Allah accept this effort and make it of real benefit to all who read it.

Salah Eddine

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to my fiance Kamel.

I also pay thankfulness to my best friends; Samia, Sihem, Dounia, Nadjet, Fatima , Meriem and Sabrina.

Without forgetting chicks family: Nesrine, Meriem, Hamada, Imene, Amine, Raouf, Imed, Badro, Diyaa, Ala elrahmen and Aya.

Finally I pray that Allah accept this effort and make it of real benefit to all who read it.

FATIMA

ACKNOWLEDGMENTS

First of all we would like to express our thanks and sincere gratitude to our academic advisor **Dr. Khedara Abdelkrim** for his countless hours of reflecting, reading, encouraging and most of all patience throughout the entire process, we are profoundly grateful to him.

Further appreciation and thankfulness are extended to **Dr. Nouadri Tahar** and **Mme. Boukhalfa Hayat** for agreeing to serve on our committee and for their valuable suggestions,which contributed to the improvement of this work.

Finally we would like to thank the staff members of the Faculty of natural sciences and life, University of Constantine1 for their continued support.

TABLE OF CONTENTS

TABLE OF CONTENTS

ABREVIATIONS

ADP: Adenosine diphosphate

ATP: Adenosine triphosphate

AP: Aminophenazone

CHE: Cholesterol esters

CHOD: Cholesterol oxidase

CMG: Chios mastic gum

DAP: Dihydroxyacetone phosphate

GAL: Galactomannans

GOD: Glucose oxidase

GPO: Glycerol phosphate dehydrogenase

GK: Glycerol kinase

G3P: Glycerol-3-phosphate

HDL: High-density lipoproteins

H2O2: Hydrogen peroxide

LDL: Low density lipoprotein

LPL: Lipoproteinlipase

PL: Pestacialentiscus

POD: Peroxidase

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

Developing countries are using herbal drugs and it became the spine of about 75–80% of the world's population, for treatment of lot of diseases due the compatibility with the human body and producing lesser side effects. Approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant chemical constituent such as Atropine (Choudhary *et al.,* 2009).

Pistacia lentiscus is an aromatic tree of the family Anarcadiaceae. It has a long history and a medicinal herb. The higher parts of *Pistacia lentiscus*is traditionally used to treat hypertension and possesses stimulant and diuretic properties (Bentley *et al.,* 1980). The resin part which is called mastic tree has many medicinal value, it is used in traditional medicines like Unani and Ayurveda system (Palevitch *et al*., 2000). and used as chewing gum, against lip-dryness, some stomach diseases, it is also used as antiseptic for respiratory system (Baytop, 1999; Tuzlaci *et al*., 2001). Essential oils of *Pistacia lentiscus* have been traditionally used for the treatment of infections and diseases all over the world for centuries (Rios and Recio, 2005).

Multiple studies have been reported on the chemical composition of essential oil of *Pistacia lentiscus* belonging to different regions in the world (De Pooter *et al*., 1991; Lo Presti *et al*., 2008). *PL* are very rich in tannins and flavonoids and glactomannan (Hamad *et al*., 2011; Bendifallah *et al*., 2014). which facilitate weight loss and prevent gain body fat (Meydani *et al*., 2010). Glactomannan have the ability to inhibit pro-inflammatory cytokines (Badia *et al.,* 2012). and decrease body weight (Srichamroen *et al., 2008).*

Obesity is a clinical term for excess body fat used in traditional medical model. It is generally applied to a person who is 20% to 30% or more above. Obesity is associated with numerous health problems, including hyperlipidemia, carbohydrate intolerance, cancer, coronary heart disease, pulmonary and renal problems, pregnancy complications, diabetes and hypertension (Haslam et al., 2005). Self imposed diets and exercise are the most frequently used methods for weight control. The role of exercise is increasing energy expenditure and regulating food intake. Low-fat diets have been shown to be effective in promoting weight loss. A food field research that has recently aroused considerable interest

is the potential of natural products to counteract obesity. Studies using different compounds of *PL* oil such as Flavonoids, Galactomannan have successfully elucidated several physiological roles of these compounds on obesity, hypolipidemia, diabetes and oxidative stress. (Srichamroen *et al., 2008 ;* Meydani *et al***.,** 2010; Pamhidzai *et al*., 2014 ; Wang *et al*., 2014).

Taking all these previous studies in considerations, both of obesity and *PL* seems to be associated with the same diseases such as cancer, diabetes. However little is known about the effect of *PL* oil on body fat. This study was conducted to see the effect of 5% *Pistacia lentiscus* oil on the body fat. Here we elucidated the first evidence that feeding rats 5% *Pistacia lentiscus* oil caused lower body fat.

BRIEF REVIEW ON *Pistacia lentiscus*

BRIEF REVIEW ON *PISTACIA LENTISCUS*

1. BRIEF REVIEW ON *Pistacia lentiscus* oil:

1.1 : *Pistacia lentiscus*

1.1.1. Definition

Pistacia lentiscus is an evergreen shrub can reach 3 m in height and grows in many countries (**figure 1)**. It belongs to the family *Anacardiaceae*

Taxonomical classification of *Pistacia lentiscus (*Nahida and saddiqui, 2012).

Kingdom :*Plantae*
Division :*Magnoliophyta*
Order :*Sapindales*
Family :*Anacardiaceae*
Genus :*Pistacia*
Species :*Pistacia lentiscus*
Binomial name: *Pistacia lentiscus*

Botanical Description

Pistacia lentiscus is an aromatic annual shrub or small tree it belong to the *Anacardiaceous* family (Lauk *et al*., 1996). Commonly known as mastic tree or mastagi, It is found in the Mediterranean region (Dogan *et al*., 2003). It is a natural remedy that has been used by ancient Mediterranean civilizations (Pellecuer *et al*., 1980). It is used for many treatment like eczema, diarrhea, throat infections and as a potent antiulcer agent (Shtayeh *et al*., 1998). It is also used in the treatment of hypertension and possess stimulant and diuretic properties (Bently *et al*., 1980). The mastic gum has been used for the relief of upper abdominal discomfort, stomachaches, dyspepsia and peptic ulcer (Al-Habbal *et al*., 1984). The essential oil extracted from the aerial parts has been proven to exhibit antioxidant, anti-inflammatory, antimicrobial (Benhammou *et al*., 2008), antifungal (Duru *et al*., 2003; Kordali *et al*., 2003). and antiatherogenic activities (Dedoussis *et al*., 2004). Medicinal virtues of the fatty fruit's oil are particularly known in North Africa, in the eastern region of Algeria and Tunisia. The people of these regions have used this fruit's oil externally to treat sore throats, locally to remedy burns and wounds and internally for respiratory allergies (Benhammou *et al*., 2008).

Figure 1: ***Pistacia lentiscus*** (Belfadel, 2009).

1.1.2. *Pistacia lentiscus* composition oil

Given the limitation of therapeutic chemicals drugs the development of research on medicinal plants has been directed towards the achievement of herbal medicines, each plant has specific constituent (Bendifallah *et al*., 2014). The essential chemical constituent of *PL* are fatty acids such as oleic acid, linoleic acid, palmitic acid and stearic acid (Charef *et al*., 2008). In addition to phenol compounds like flavonoids, enthocyanin, tannins and glycoside (Bendifallah *et al*., 2014), and galactomannan (Hamad *et al*., 2011).

1.1.2.1 Flavonoids

Flavonoids are synthesized by plants, and their presence in animals tissues strictly depends on the intake of plant products (Kyle *et al.,* 2006). It is noteworthy that flavonoids are often less toxic and reveal lower side effects than derivatives produced from other natural compounds. Nevertheless, similar to any chemical, flavonoids can be harmful at high doses (Ebrahimi *et al*., 2012). Variety of flavonoids found in the nature possesses their own physical, chemical, and physiological properties. Structure function relationship of flavonoids is epitome of major biological activities. Medicinal efficacy of many flavonoids as antibacterial, hepatoprotective, anti-inflammatory, anticancer, and antiviral agents is well established (Shashank *et al*., 2013).

1.1.2.2 Galactomannan

Galactomannans (GAL) are a group of storage polysaccharides from plant seeds that reserve energy for germination in the endosperm. It is mucilaginous fiber (Prajapati *et al.* 2013). Previous studies showed that the fiber galactomannan has the potential to modifying both glycemic and lipidemic status as well as body weight in rats (Srichamroen *et al., 2008).* Also, galactomannan component from many other plants showed ability in inhibiting the pro-inflammatory cytokines (Badia *et al.*, 2012).

1.2 Pharmacological aspects and therapeutic effects of *Pistacia lentiscus*

Pistacia lentiscus is known for its medicinal properties since antiquity (Palevitch *et al*., 2000). The decoction of dried roots is effective against intestinal inflammation and stomach as well as in the treatment of ulcer (Ouelmouhoub, 2005). The aerial part of *Pistacia lentiscus*is widely used in traditional medicine in the treatment of high blood pressure due to its diuretic properties (Bentley *et al*., 1980; Sanz *et al*., 1992; Scherrer *et al*., 2005). The leaves are provided with anti- inflammatory, antibacterial, antifungal, antipyretic, astringent, hepatoprotective, expectorant and stimulating (Magiatis *et al*., 1999; Janaka *et al*., 2002; Kordali *et al*., 2003; Paraschos *et al*., 2007). They are also used in the treatment of other diseases such as eczema, mouth infections, diarrhea, kidney stones, jaundice, headaches, ulcers, sore stomach, asthma and respiratory problems (Shtayeh *et al*., 2000; Said *et al*., 2002). The resin obtained from *Pistacia lentiscus* is known for its analgesic, antibacterial, antifungal, anticancer, antioxidant, antithérogenique, expectorant, stimulant, diuretic and spasmolytic (Magiatis *et al*., 1999; Dedoussis *et al*., 2004; Prichard, 2004; Assimopoulou *et al*., 2005).

1.2.1. Antioxidant activity of *Pistacia lentiscus*

It has been reported that *PL* has antioxidant property due to digallic acid which has ability to scavenge the free radical. Galloylquinic acid isolated from leaves of this plant have has also antioxidant property because it reduce strongly the oxidation of LDL (Liubuncic *et al*., 2005). Also it is well known that *PL* has the ability to supress the extent of iron induced lipid peroxidation in rat liver homogenate (Nahida and Saddiqui, 2012).

1.2.1. Anticancer activity of *Pistacia lentiscus*

Balan *et al., (*2007) reported that 50% ethanolic extract of chios mastic gum (CMG) of *PL* inhibits proliferation. Dimas *et al., (2009)* also reported that hexane extract of mastic gum is also used in the treatment of colorectal tumors. Merilan He *et al.,(2006)* reported that gum mastic was used to treat prostate cancer.

1.2.2. Antimicrobial activity

Essential oil from aerial parts which contain terpineol and α-terpineol is effective against mycelian growth of *Aspergilus flavus (*Benhammou *et al*., 2008). Literature reports that the leaf Extract of *Pistacia lentiscus* was tested for antimicrobial property and it has been observed that it has a strong antifungal but weak antibacterial activity (Iank *et al*., 1996). In another study, *PL* has antimycotic activity (Magiatis *et al*., 1999). Its essential oil which is obtained from leaves, twigs and mastic gum showed in vitro antimicrobial activity and antifungal activity against *rhizoctania solani (*Raffaele *et al*., 2002). It's aqueous and flavonoid enriched extract and essential oil from leaves has marked inhibitory effect against *Salmonella typhimurium* and lower inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella enteritidis (*Aksoy *et al., 2006)*. It has been illustrated that the acetone extract of *Pistacia lentiscus* has more significant antibacterial activity against *S.Mutans*and *Mutans streptococci* in vitro and in vivo (Tassou *et al*., 1995). Essential oil from mastic gum is also effective against Gram positive and Gram negative bacteria (Pal *et al*., 2009).

MATERIALS AND METHODS

2. Materials and Methods

2.1 Animals and diets

Male Albino Wistar rats weighing 126g were used. Rats were individually housed in plastic cages. Room temperature was kept at 24 C on a 12 h light-dark cycle (lights on, 08:00-20:00 h). All rats were given free access to water. Composition of the basal diet is shown in **Table** 1. All animals were fed the same amount of experimental diets (12 g d1,14g d2,16g d3-4,17g d5,18g d6,19g d7 and 20g for d 8-28). All the diets were daily incorporated into food cups in the cages at 10:00h; and all rats completely consumed the diets until the next morning. After 28 days of consuming diets, food was removed from the cages at 08:00 h and the rats were lightly anesthetized with chloroform and killed between 11:00 and 14:00 h.Blood was collected and samples were allowed to clot on ice. Serum samples were obtained by centrifugation (3000 rpm for 10 min). abdominal adipose tissues (epididymal and perirenal adipose tissues) were immediately removed, weighed and stored at -80 C until use.

2.2 Experiment

Rats were divided into two groups of 6 rats each, control group (C) and *Pestacia lentiscus* diet group (PL).

Table 1 Composition of basal diets [1]

Ingredients	**Control group**	**PL group**
	% (W/W)	
DL-Methionine	0.3	0.3
Vitamin mixture	1.0	1.0
Salt mixture	3.5	3.5
Casein	20	20
Corn oil	5.0	0
Sucrose	21.7	21.7
Cellulose powder	5	5
α-Corn starch	43.5	43.5
PL	0	5.0

[1]Diets are modified version of AIN J. Nutr. 107: 1340-1348 (1977).

2.3 Analytical procedure

Serum concentrations of glucose, total cholesterol and albumin, triglycerides, HDL cholesterol and creatinine were measured in the laboratory of biochemistry, Polyclinique Debahi Louiza. Grarem, Mila using kits (BIOMEGHREB; TUNISIE SPINREACT; SPAIN, TECO; CALIFORNIA, BIOSYSTEMS; SPAIN, QCA: Quimica Clinica Aplicada; SPAIN) respectively.

2.3.1 Quantitative determination of glucose

a. Test principe of glucose

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed hydrogen peroxide (H2O2) is detected by a chromogenic oxygen acceptor, phenol, 4 –aminophenazone (4-AP) in the presence of peroxidase (POD):

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample

b. Procedure:

One milliliter of the reaction mixture containing 92 mM TRIS buffer (pH 7.4), 0.3 mM phenol, 15000 U/L glucose oxidase (GOD), 1000U/L peroxydase (POD) and 2.6 mM 4-aminophenazone (4-AP) was incubated with10 µl sample. After incubation at 37ºC for 10 min, optical density of sample and standard (Glucose aqueous primary standard 100 mg/dl) were recorded against blank. At the wavelength 505nm using Spectrophotometer (MINDRAY. BA-88A).

The concentration of serum glucose was calculated by the difference in absorbance between the standard and the sample (Eq. 1) and (Eq.2)

(A) Sample / (A) Standard X 100 (Standard conc.) = mg/dl glucose in the sample (Eq.1).

Then mg/dl X 0.0555 (conversion factor) = mmol/L (Eq.2).

2.3.2 Quantitative determination of Triglycerides

a. Test principe of triglycerides

Sample triglycerides incubated with lipoproteinlipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase (GK) and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2). In the last reaction, hydrogen peroxide (H2O2) reacts with 4-aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye:

$$\text{Triglycerides} + HO_2 \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-AP} + \text{p-chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + 4H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample.

b. Procedure

One milliliter of working reagent contain 50 mM GOOD buffer (pH 7.5), 2 mM 4-chlorophenol, LPL 150000 U/L, GK 500 U/L, GPO 2500 U/L, POD 440 U/L, 4-AP 0.1 mM and 0.1 mM ATP was incubated with10 µl sample. After incubation at 37ºC for 10 min, optical density of sample and standard (Glucose aqueous primary standard 100 mg/dl) were recorded against blank. At the wavelength 505nm using Spectrophotometer (MINDRAY. BA-88A).

The concentration of serum triglycerides was calculated by the difference in absorbance between the standard and the sample (Eq. 3) and (Eq.4)

(A) Sample / (A) Standard X 200 (Standard conc.) = mg/dl triglycerides in the sample (Eq.3).

Then mg/dl X 0.0113 (conversion factor) = mMol/L (Eq.4).

2.3.3 Quantitative determination of cholesterol

a. Test principe of cholesterol

The cholesterol present in the sample originates a colored complex, according to the following reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{CHE} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{4-Cholestenona} + H_2O_2$$

$$2\,H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{POD} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

b. Procedure:

One milliliter of the reaction mixture containing 90 mM PIPES buffer (pH 6.9), 26 mM phenol, 1000 U/L Cholesterol esterase (CHE), 300U/L Cholesterol oxidase (CHOD) , 650 U/L Peroxidase (POD) and 0.4 mM 4 – Aminophenazone (4-AP) was incubated with10 µl sample. Each sample had both blank and standard (Cholesterol aqueous primary standard 200 mg/dl). After incubation at 37ºC for 10 min, optical density at the wavelength 505 nm was recorded using Spectrophotometer (MINDRAY. BA-88A). The concentration of serum cholesterol was calculated from the difference in absorbance between the standard and the sample (Eq. 5) and (Eq. 6).

(A) Sample / (A) Standard X 200 (Standard conc.) = mg/dl cholesterol in the sample (Eq. 5).

Then mg/dl X 0.0258 (conversion factor) = mMol/L (Eq. 6).

2.3.4 Quantitative determination of HDL-Cholesterol

a. Test principe of HDL-Cholesterol

Directly determination of serum HDLc (high-density lipoprotein cholesterol) levels without the need for any pre-treatment or centrifugation of the sample.

The assay takes place in two steps.

1º Elimination of lipoprotein no-HDL

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2\ H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

2º Measurement of HDL-c

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenona} + H_2O_2$$

$$2\ H_2O_2 + \text{HDAOS} + \text{4-AP} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the HDLc concentration in the sample**.**

b. Procedure

Two reagents the first one is R1 for elimination of lipoprotein no-HDL , 300 microlitter contain N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid (pH 6.6) 100 mM , N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline (HDAOS) 0.7 mM, Cholesterol Esterase ≥ 800 U/L, Cholesterol oxidase≥ 500 U/L, Catalase ≥ 300 U/L Ascorbic oxidase ≥ 3000 U/L was incubated with 3 µl sample for 5 min at 37ºC. The absorbances (A1) of the samples and standard (Lyophilized human serum) were recorded against blank using automate (MINDRAY.BA-88A) at the wavelength 600 nm.

Then adding 100 µl of the second reagent R2 contain N,N-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid pH 7.0 100 mM , 4 – Aminoantipyrine (4-AP) 4 mM, Peroxidase ≥ 3500 U/L and incubate for 5 min at 37ºC again. After that reading the absorbance (A2) of the samples and calibrator, against the blank and calculate the increase of the absorbance ΔA= A2 – A1.

The concentration of serum HDL-Cholesterol was calculated from the difference in absorbance between the standard and the sample (Eq. 7) and (Eq. 8).

(ΔA) Sample / (ΔA) Standard X (Standard conc.) = mg/dl HDL-Cholesterol in the sample (Eq. 7). Then mg/dl X 0.0259 (conversion factor) = mmol/L (Eq. 8).

2.3.5 Quantitative determination of albumin

a. Test principe of albumin

Albumin in the presence of bromcresol green at a slightly acid pH, produces a colour change of the indicator from yellow-green to green-blue. The intensity of the color formed is proportional to the albumin concentration in the sample.

b. Procedure

One milliliter of the reaction mixture containing 0.12 mM Bromcresol green buffer (pH 4.2) was incubated with 5 µl sample. Each sample had both blank and standard (Albumin aqueous primary standard 5 g/dl). After incubation for 10 min at room temperature (15-25 °C) optical density at the wavelength 630 nm was recorded using Spectrophotometer (MINDRAY. BA-88A). The concentration of serum albumin was calculated from the difference in absorbance between the standard and the sample (Eq. 9) and (Eq. 10).

(A)Sample / (A) Standard X 5 (Standard conc.) = g/dl albumin in the sample (Eq. 9).

Then g/dl X 144.9 = µmol/L (Eq. 10).

2.3.6 Quantitative determination of Creatinine

a. Test principe of creatinine

At an alkaline pH, creatinine reacts with picrate to form a Janovskycomplex.The rate of increase in absorbance at 492 nm due to the formation of the creatinine-picrate complex is directly proportional to the concentration of creatinine in the sample.

b. Procedure

A Creatinine Base Reagent containing 0.29 mol/L sodium hydroxide and surfactants and a Creatinine Picrate Reagent containing 17.5 mM picric acid can be used separately on two reagent systems. A working reagent may be prepared by combining one volume of creatinine picrate reagent and four volumes of creatinine base reagent. Mix well before using.

Studies using this reagent were performed on an automated analyzer using a kinetic test mode, with a sample to reagent ratio of 1:24, and a wavelength reading of 505 nm. The analyzer automatically calculates the creatinine concentration of each sample.

2.4 Statistical analysis

All results were tested for statistical significance by Student's t-test using StatView softwear.

RESULTS AND DISCUSSION

a. effect of 5% *Pistacia lentiscus* on Final body weight

There was no significant difference in final body weight between the two groups (Fig. 2). Pervious studies showed that *Pistacia lentiscus* has no effect on the final body weight (Djerrou *et al*., 2011) and that is in agreement with our study.

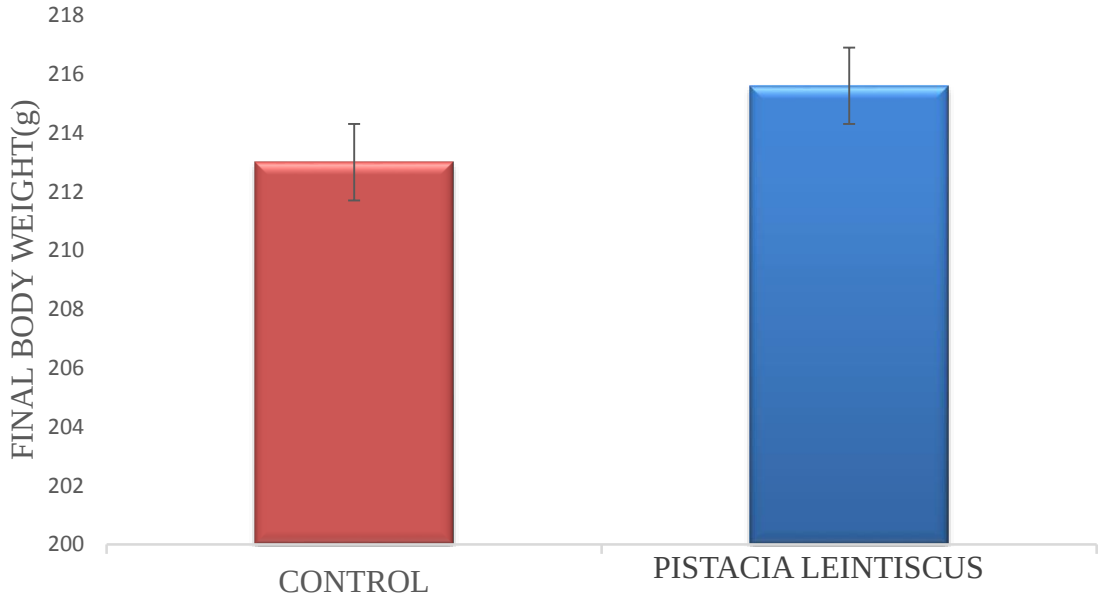

Figure2 : effect of 5% *Pistacia lentiscus* on Final body weight[1]

1 Value are means ± SE (n=6) for each group.

b. effect of 5% *Pistacia lentiscus* on serum Albumine and Creatinine

The concentrations of albumine and creatinine which are indicators of renal function were not affected by 5% traitement of *Pistacia lentiscus*. and the renal function is not alliterate(Fig. 3,4). A number of observational studies showed that lipid abnormalities are associated with a failure in kidney function. Hyperlipidemia is associated with hyperlipoproteinemia and Chylomicronemia syndrome (Mahamuni *et al*., 2012). recent clinical studies in patients with the nephrotic syndrome have suggested that reduction of hyperlipidemia may be associated with a decreased proteinuria and improvement in renal function (Reblink *et al*., 1990, Suzaki *et al*., 1990). in the present study the dietary addition of 5% Pistacia lentiscus does not affect albumine and creatinine. Therefore the hypolimedimia induced by 5% *Pistacia lentiscus* is not due to renal dysfunction.

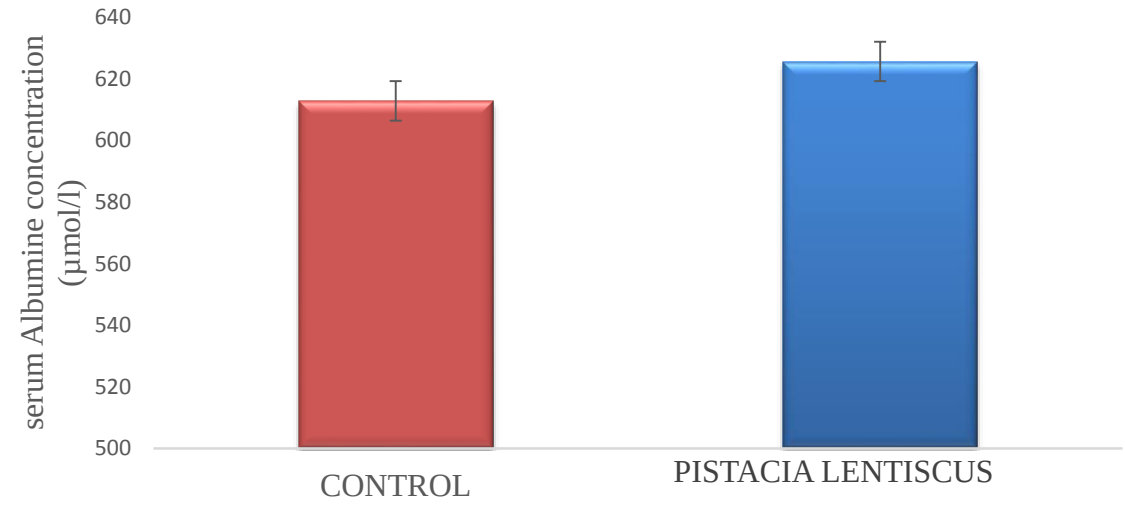

Figure 3: effect of 5% *Pistacia lentiscus* on serum Albumine[1]

[1] Value are means ± SE (n=6) for each group

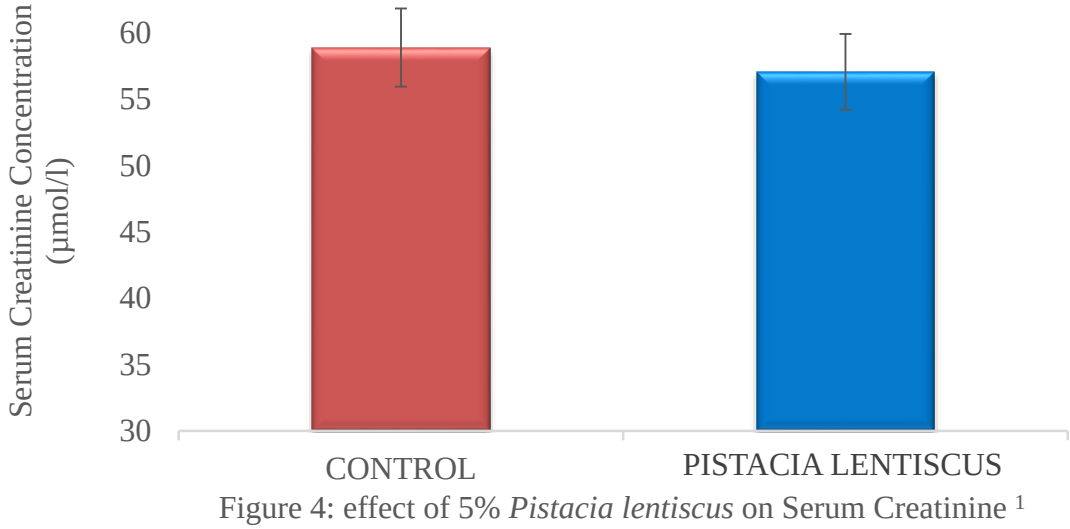

Figure 4: effect of 5% *Pistacia lentiscus* on Serum Creatinine [1]

1 Value are means ± SE (n=6) for each group

c. effect of 5% *Pistacia lentiscus* on Triglyceride, Cholesterol, HDL and LDL

The addition of 5% *Pistacia lentiscus* significantly caused 34% decreasing in serum triglyceride without affecting serum total cholesterol, HDL and LDL cholesterol ($P < 0.05$, Fig. 5, 6, 7 and 8). From these results, we speculate that addition of 5% *Pistacia lentiscus* may activates hepatic fatty acid oxidation, which leads to elevated conversion of fatty acid to ketone bodies, thereby decreasing serum triglyceride. (Further study is needed to measure serum free fatty acid and the activity of hepatic carnitine palmitoyltransferase, a rate limiting enzyme of mitochondrial fatty acid oxidation). It has been reported that feeding *Pistacia lentiscus* caused hypotriglyceridemia and hypocholesterolemic (Djerrou *et al*., 2014; Cheurfa *et al.*, 2015). Our results are in agreement with these studies. However, the unchanged total serum cholesterol, HDL and LDL cholesterol in our study it may due to the feeding period (Further study is needed to extend the feeding period).

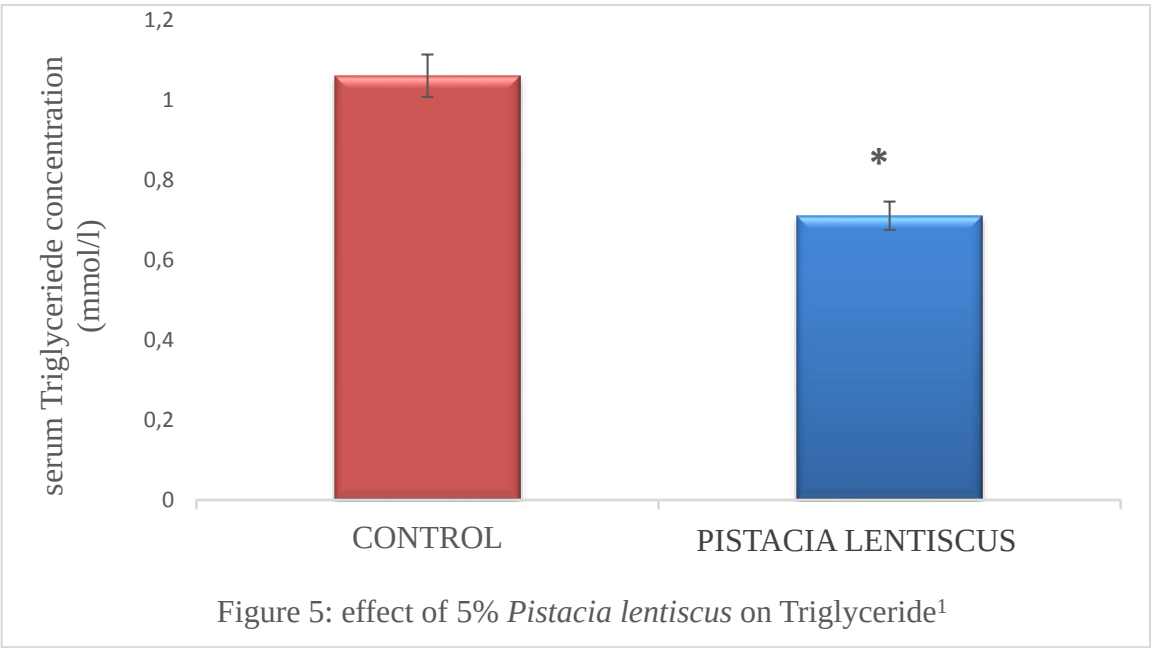

Figure 5: effect of 5% *Pistacia lentiscus* on Triglyceride[1]

[1] Value are means ± SE (n=6) for each group

*Significantly different from control by student's test ($P < 0.05$)

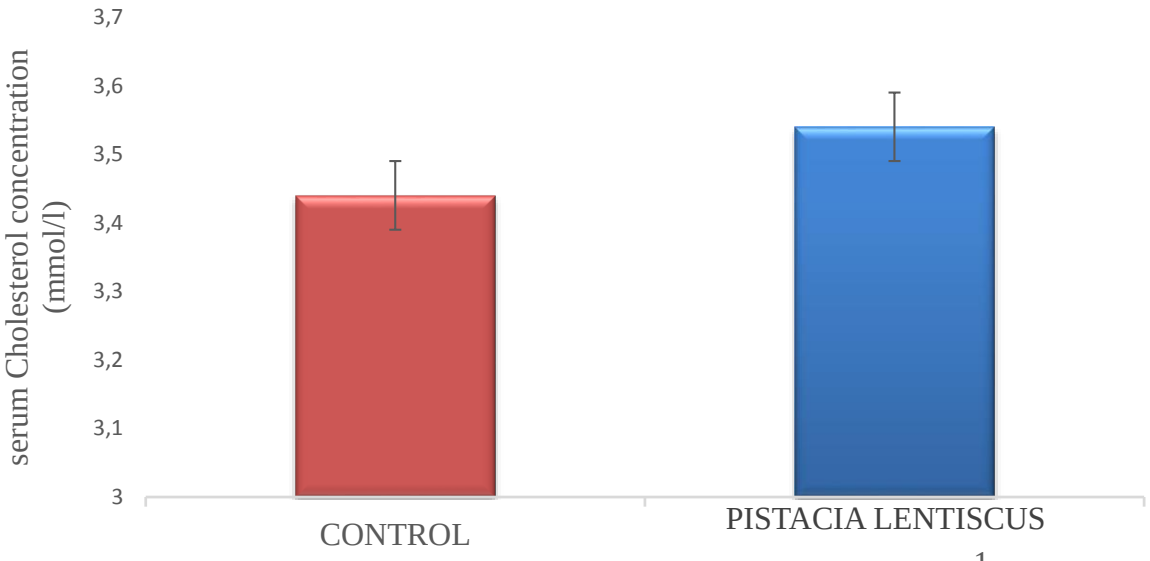

Figure 6: effect of 5% *Pistacia lentiscus* on serum Cholesterol [1]

[1] Value are means ± SE (n=6) for each group

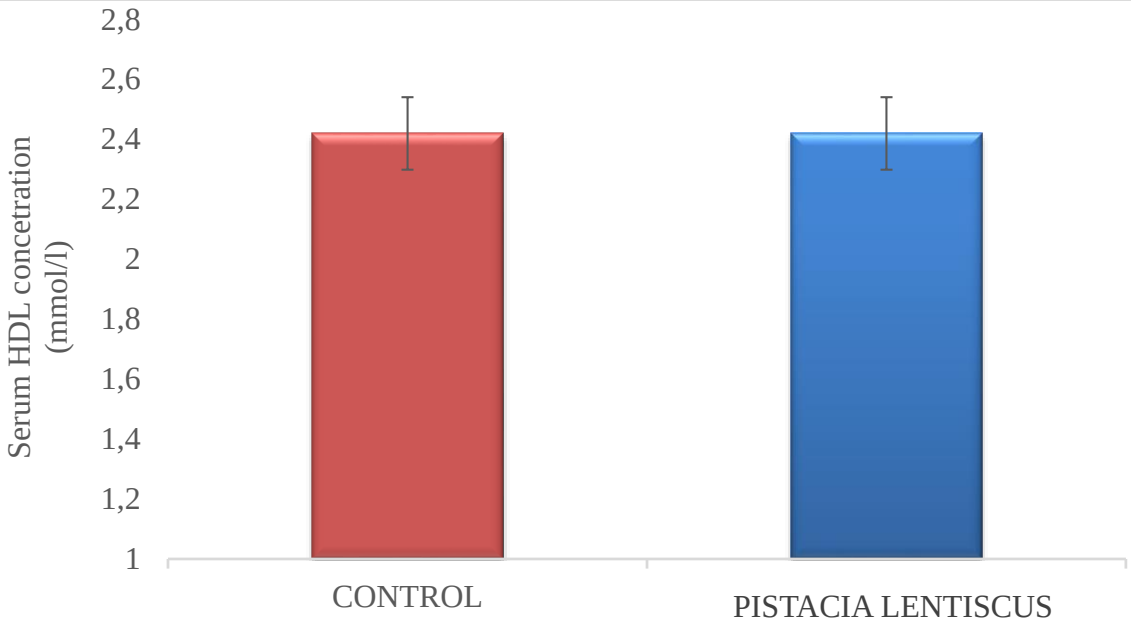

Figure 7: effect of 5% of *Pistacia lentiscus* on HDL Concentration[1]

[1] Value are means ± SE (n=6) for each group.

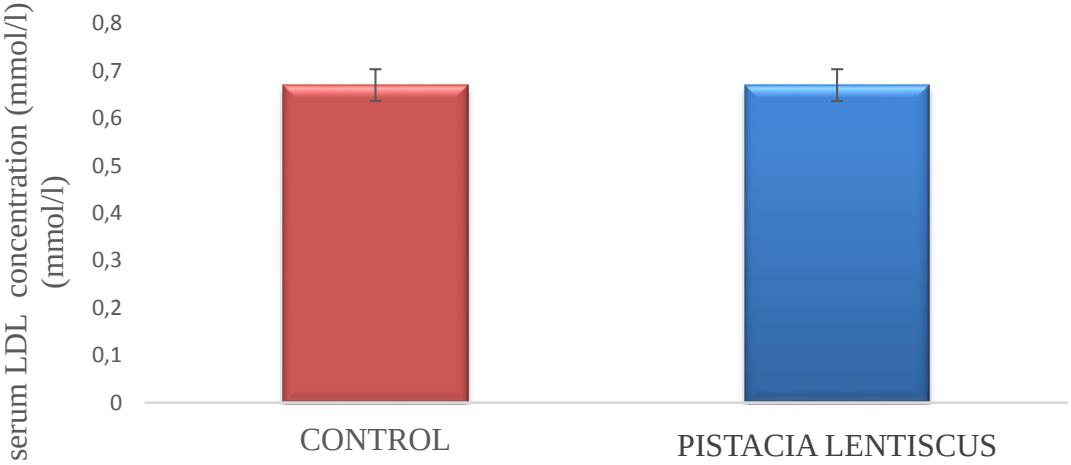

Figure 8: effect of 5% *Pistacia lentiscus* on LDL concentartion [1]

[1] Value are means ± SE (n=6) for each group

d. effect of 5% *Pistacia lentiscus* on Serum Glucose

Serum glucose concentration was slightly reduced by 5% *Pistacia Lentiscus* treatment (Fig. 9). Our results are in agreement with the previous studies. It has been shown that *Pistacia Lentiscus* feeding caused lower serum concentration of glucose (Angeliki *et al*., 2006).

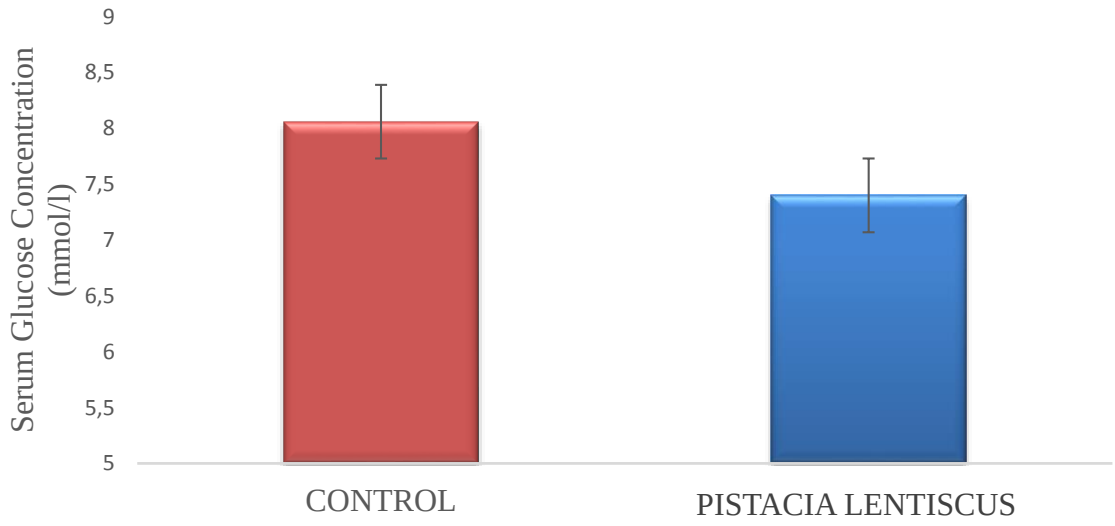

Figure 9 :effect of 5% *Pistacia lentiscus* on Serum Glucose [1]

[1] Value are means ± SE (n=6) for each group.

e. effect of 5% *PISTACIA LENTISCUS* on relative Perirenal and Epididymal adipose tissue weight[1]

The results showed that 5% *Pistacia Lentiscus* feeding significantly caused 23% reduction in relative epididymal adipose tissue weight. However, Perirenal adipose tissue was slightly reduced by 5% *Pistacia Lentiscus* treatment ($P < 0.05$, Fig. 10 and 11). polyphenols and Galactomannan are known to prevent obesity (Meydani *et al*.,2010; Ramulu *et al*., 2011). *Pistacia Lentiscus* and its compounds such as polyphenols and Galactomannan are well known to act as antioxidant nutrients (Pamhidzai *et al*., 2015). *Pistacia Lentiscus* has antitumor and antibacterial activities (Benhammou *et al*., 2008; Remila *et al*., 2015).

. Mortality from all cancers and the incidence rates of several specific types of cancer are elevated in overweight individuals (Lew and Garfinkle, 1979). In view if these facts, we speculate that obesity and cancer might be at least in part accounted for by a common mechanism involving *PL* or its compounds as antioxidant nutrients.

In consistent with hypotriglyceridemic effect of *PL*, we speculate also that higher fatty acid oxidation due to the activation of carnitine palmitoyltransferase is responsible for the lower accumulation of body weight by *Pistacia Lentiscus.*

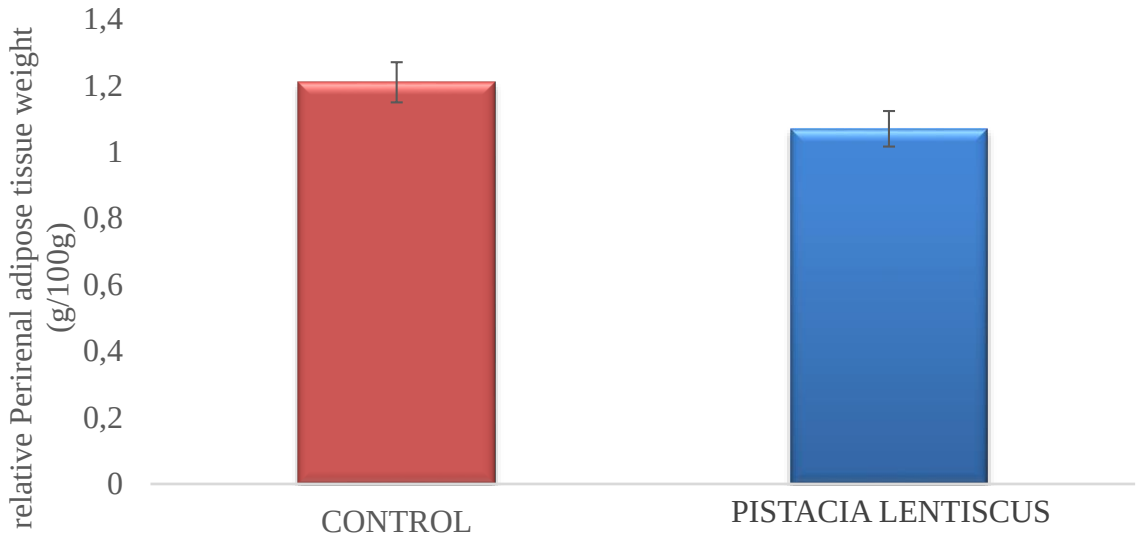

Figure 10: effect of 5% *Pistacia lentiscus* on relative Perirenal adipose tissue weight[1]

[1] Value are means ± SE (n=6) for each group

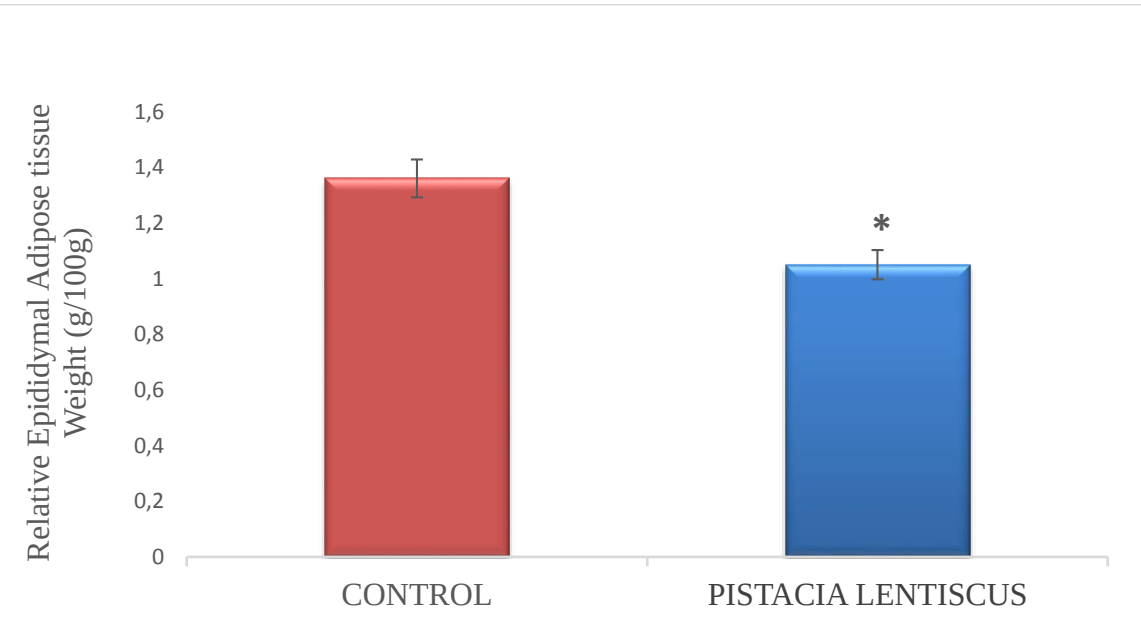

Figure 11:effect of 5% *Pistacia Lentiscus* on Relative Epididymal Adipose tissue Weight[1]

[1] Value are means ± SE (n=6) for each group

*Significantly different from control by student's test ($P < 0.05$).

CONCLUSION AND PERSPECTIVE

CONCLUSION

Obesity is a clinical term for excess body fat used in traditional medical model. It is a disease which affects more than 30% of United States adults, with a higher incidence among women (Mokdad et *al*., 2001; Ogden *et al*., 2006).obesity is a major risk factor for diabetes, cardiovascular diseases, several forms of cancer, pulmonary, osteoarticular and metabolic diseases (Bronson *et al*., 1999; Kenchaiah *et al*., 2002; Calle *et al*., 2003).

This study provided the first evidence that feeding rats 5% *Pistacia lentiscus* oil caused not only hypotriglyceridemia but also lower body fat. The results suggest that 5% *Pistacia lentiscus* oil activates fatty acids oxidation, which leads high conversion of fatty acids to ketone bodies, thereby reducing serum triglyceride. This phenomena is one of the mechanisms responsible for the lower accumulation of body fat.

Studies were reported that The active compounds of *Pistacia lentiscus* such as polyphenols and galactomannan lower food intake, decrease lipogenesis, increase lipolysis, stimulate fatty acids oxidation, inhibit adipocyte differentiation and growth, attenuate inflammatory responses and suppress oxidative stress (Wang *et al*., 2014; Pamhidzai *et al*., 2015).

In summary, this study has demonstrated lower body fat and hypotriglyceridemia caused by feeding rats 5% *Pistacia Lentiscus* oil and suggest that the reduction in body fat is mediated by the activation of the hepatic fatty acid oxidation. This study further raises an important question whether some nutrients like *Pistacia Lentiscus* acting as antioxidant can also affect body fat accumulation.

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SUMMARY

Summary

Pistacia lentiscus is an evergreen shrub belongs to Anarcadiaceae family. It contains polyphenols and galactomannan. These compounds make it a potent medicinal herb that used for treatment of many diseases such as hypertension, lip-dryness, some stomach diseases, infections, dyslipidemia, diabetes and cancer. Obesity is medical condition, it is considered as a major threat to the public's health. it facilitates the metabolic disorders such as cardiovascular diseases, diabetes, hypertension and cancer. From these previous studies, obesity and *Pistacia lentiscus* seems to be associated with the same diseases such as cancer, diabetes and suggest a possible effect of *Pistacia lentiscus* on obesity.

This study was conducted to define the effect of 5% *Pistacia lentiscus* on serum lipid and body fat accumulation. Rats were fed for 28 days diets with or without 5% *Pistacia lentiscus*. Feeding a diet containing 5% *Pistacia lentiscus* caused 23%, 12% reduction in relative epididymal and perirenal adipose tissue weight respectively, 34% decreasing in serum triglyceride and 5 % decreasing in serum glucose concentration.

This study provided the first evidence suggesting that 5% *Pistacia lentiscus* lower body fat accumulation. *Pistacia lentiscus* appears to have anti-obesity effect by inhibiting adipocyte differentiation through a mechanism involving its active compounds such as polyphenols and galactomannan.

***Keywords*: *Pistacia lentiscus*, Hypocholesterolemia, Hypotriglyceridemia, Obesity**

Résumé

Résumé

Lentisque pistachier est un arbuste à feuilles persistantes qui appartient à la famille Anarcadiaceae. Il contient des polyphénols et galactomannane. Ces composés font une plante médicinale puissante utilisée pour le traitement de nombreuses maladies telles que l'hypertension, la sécheresse des lèvres, certaines maladies de l'estomac, les infections, la dyslipidémie, le diabète et le cancer. L'obésité est un état considéré comme une menace majeure pour la santé. Elle facilite les troubles métaboliques tels que les maladies cardiovasculaires, le diabète, l'hypertension et le cancer. De ces études, l'obésité et le *Lentisque pistachier* semble être associée avec les mêmes maladies telles que le cancer, le diabète et suggèrent un effet possible de *Lentisque pistachier* sur l'obésité.

Cette étude a été menée pour définir l'effet de 5% de Lentisque pistachier sur les lipides et l'accumulation de graisse dans le corps. Les rats ont été nourris pendant 28 jours avec et sans cette dernière. L'alimentation d'un régime contenant cette dose de notre plante a causé 23% de réduction du tissu adipeux de l'épididyme et 12% du tissu adipeux périrénales ainsi que 34% des triglycérides en plus de 5% de la concentration de glucose dans le sérum.

Cette étude a fourni la première preuve suggérant que 5% *Lentisque pistachier* diminue l'accumulation de graisse corporelle. *Lentisque pistachier* semble avoir un effet anti-obésité en inhibant la différenciation des adipocytes par un mécanisme impliquant ses composés actifs tels que des polyphénols et galactomannane.

***Mots-Clés* : *Pistacia lentiscus*, Hypocholestérolémie, Hypotriglycéridémie, L'obésité.**

ملخص

البطم العدسي(شجرة زيت الضرو) شجيرة دائمة الخضرة تنتمي إلى أسرة أناركادياسيي. تحتوي على متعدد الفينول وجالاكتومانان. هذه المركبات تجعلها من الاعشاب الطبية الفعالة. يستعمل زيت البطم العدسي في معالجة الكثير من الأمراض مثل ارتفاع ضغط الدم وجفاف الشفاه، بعض أمراض المعدة، التهابات، تعديل نسبة الدهون، السكري والسرطان. تعتبرالسمنة حالة مرضية تهدد الصحة العامة. فهي تسهل الاضطرابات الأيضية مثل أمراض القلب، الأوعية الدموية، السكري وارتفاع ضغط الدم . من المعطيات السابقة هناك علاقة ممكنة بين السمنة و زيت البطم العدسي حيث يرتبطان مع نفس الأمراض مثل السرطان والسكري، والنتيجة إمكانية تأثير زيت البطم العدسي على السمنة.

أجريت هذه الدراسة لتحديد تأثير 5% من زيت البطم العدسي على البدانة و ايض الدهون عند الفئران. تم تغذية الفئران لمدة 28 يوما تغذية أساسية ب أو بدون 5% من زيت البطم العدسي. النظام غذائي الذي يحتوي على 5% من زيت البطم العدسي تسبب في انخفاض معنوي بنسبة 23 ٪ و12% في النسيج الدهني البطني و النسيج الدهني الدهني المحيط بالكلى على التوالي، وكذلك انخفاض بنسبة 34% و5% في **الجليسريدات الثلاثية** والجلوكوز **في** الدم.

والخلاصة، تعتبرهذه الدراسة أول دليل يبين بأن تناول 5% من زيت البطم العدسي يكون له تأثير معاكس للبدانة من خلال تاثير المكونات الفعالة لزيت هذه الشجرة مثل البوليفينولات والجالاكتومانان.

الكلمات المفتاحية :زيت البطم العدسي ، الكولسترول، الجليسريدات الثلاثية ، السمنة.

LAIEB Salah Eddine

BOUBLIA Fatima Zohra

Presented on June 30th 2015

Effect of Dietary Addition of 5% *Pistacia lentiscus* on Serum Lipids and Body Fat in Rats

Diplôme : To Get a Diploma of Master in (Fondamental Biochemistry)

Option: Proteomic Analysis and Health

Pistacia lentiscus is an evergreen shrub belongs to Anarcadiaceae family. It contains polyphenols and galactomannan. These compounds make it a potent medicinal herb that used for treatment of many diseases such as hypertension, lip-dryness, some stomach diseases, infections, dyslipidemia, diabetes and cancer. Obesity is medical condition, it is considered as a major threat to the public's health. it facilitates the metabolic disorders such as cardiovascular diseases, diabetes, hypertension and cancer. From these previous studies, obesity and *Pistacia lentiscus* seems to be associated with the same diseases such as cancer, diabetes and suggest a possible effect of *Pistacia lentiscus* on obesity.

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***Keywords*: *Pistacia lentiscus*, Hypocholesterolemia, Hypotriglyceridemia, Obesity.**

Research laboratory: Departement of animals University brothers Constantine

certified by:	
Presedent: **Dr. T. NOUADRI**	**M. C. A University BROTHERSMENTOURI.**
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Examiner: **Dr. H. BOUKHALFA**	**M. C. B University BROTHERS MENTOURI.**