POLYSACCHARIDES AND ASCORBIC ACID CONTENT AND THE EFFECT OF AQUEOUS EXTRACT OF *Portulaca oleracea* IN HIGH-FAT DIET-INDUCED OBESITY, DYSLIPIDEMIA AND LIVER DAMAGE IN ALBINO WISTAR RATS

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Abstract.- *The aim of the present study was to evaluate the hypolipidemic and antioxidant properties of aqueous extracts of* Portulaca oleracea *in experimental obese rats. Twenty four (24) apparently healthy male Wistar rats were grouped into four groups (6 rats in each): normal, Obese, Obese + Atorvastatine (100 mg kg-1 day-1) and Obese +* P. oleracea *(400 mg kg-1 day-1.) groups. All rats except those in the control group were made dyslipidemic using a high-fat cafeteria food. All drug administration was done orally for a period of four weeks. Administration of* P. oleracea *reduced the levels of TG (26.99%), TC (10.91%) and LDL (16.41%), which were elevated by obesity. Analysis of the effect of* P. oleracea *on antioxidant liver, revealed that P. oleracea potentially regulates lipid peroxides and protects organs from oxidation-associated damage. The markers of liver damage such as SGPT and SGOT levels that were elevated ($p<0.05$) by HFD were also reduced on* P. oleracea *treatment. In conclusion, this study has shown the aqueous leaf extracts of* P. olercea*, lowers blood glucose and lipid profile levels and is a beneficial effect against the development of obesity and a good antioxidant and hepatoprotective property.*

Key words*:* Portulaca oleracea*, Polysaccharide, High Fat Diet, Obesity, liver damage .*

CONTENU DES POLYSACCHARIDES ET ACIDE ASCORBIQUE ET EFFET DE L'EXTRAIT AQUEUX DE *Portulaca oleracea* SUR L'OBESITE, LA DYSLIPIDEMIE ET LES DOMMAGES HEPATIQUES INDUIT PAR UN REGIME HYPERGRAS CHEZ LES RATS ALBINO WISTAR

Résumé.- *La présente étude cherche à évaluer les propriétés hypolipidémiates et antioxydantes des extraits aqueux de* Portulaca oleracea *chez des rats obèses expérimentaux. Vingt-quatre (24) rats Wistar mâles sont regroupés en quatre groupes (6 rats chacun): normal, obèse, obèse + atorvastatine (100 mg /kg poids) et obèse +* P. oleracea *(400 mg/ kg poids). Tous les rats, sauf ceux du groupe témoin, sont rendus dyslipidémiques en utilisant un régime cafétéria à haute teneur en matières grasses. Toute l'administration du médicament est faite par voie orale pendant une période de quatre semaines. L'administration de* P. oleracea *a réduit les taux de TG (26,99%), de TC (10,91%) et de LDL (16,41%), qui étaient élevés par l'obésité. L'analyse de l'effet de* P. oleracea *sur les antioxydants hépatiques a révélé que* P. oleracea *régule potentiellement les peroxydes lipidiques et protège les organes contre les dommages associés à l'oxydation. Les marqueurs de lésions hépatiques tels que les taux de SGPT et de SGOT qui sont élevés ($p <0,05$) par HFD ont également été réduits sur le traitement par* P. oleracea*. L'extrait aqueux de feuilles de* P. olercea*, abaisse les niveaux de glucose sanguin et de profil lipidique et a un effet bénéfique contre le développement de l'obésité et a de bonnes propriétés antioxydante et hépatoprotectrice.*

Mots clés*:* Portulaca oleracea*, polysaccharide, régime riche en graisses, obésité, dommages au foie.*

Introduction

Nutrition characterizes a lifestyle element that can be measured, and that can directly impact health; consequently defensive nutrition and weight control should develop a main focus of consumers and prepared-food providers [1]. The Westernization of diets, with an increase in obtainability of high calorie foods surely contributes to the epidemic of metabolic syndrome. Certainly, diets high in saturated fats have been revealed to induce weight gain, insulin resistance, and hyperlipidemia in humans and animals [2].Obesity has developed as one of the principle universal health problems in industrialized countries. It is associated with many diseases, principally diabetes and dyslipidemia [3]. Obesity is the major cause of the metabolic syndrome, which includes type 2 diabetes mellitus, dyslipidemia and cardiovascular disease. The classic dyslipidemia of obesity consists of augmented triglycerides, LDL-C with raised small dense LDL and reduced HDL-C [4]. Imbalance in energy produces visceral adiposity and obesity increasing reactive oxygen species (ROS) production that predisposes individuals to complications such as oxidative stress, metabolic troubles, insulin resistance, and lipid synthesis [5]. The presence of obesity rises the risk of elevated liver enzymes activities by a factor of 2 to 3, but the risk of steatosis is augmented by a factor of three in the case of overweight and a factor of around 15 in the case of obesity. Both cirrhosis and hepatocellular carcinoma are also related with obesity in the general population. In patients with nonalcoholic fatty liver disease observed in obesity and weight gain are classically associated with advanced fibrosis and fibrosis progression [6]. Phytochemicals are bioactive compounds produce in plants that work with nutrients to protect against certain diseases [7]. Some phytochemicals have antioxidant property and decrease the danger of several diseases as polyphenols and flavonoids [8]. *Portulaca oleracea* is s cosmopolitan species and the genus Portulaca belongs to the family *Portulacaceae*, a small family with 21 genera and 580 species and is cosmopolitan in distribution and occurring particularly in America with some species originate in Arabia [9]. It used as a potherb in Asian, central European and Mediterranean countries, which is utilized as one of medicinal plants and has been given term “Global Panacea” [10]. Purslane also is popular as a traditional medicine in Algeria for the treatment of various deseases. It has been reported to possess potent pharmacological actions such as hepatotprotective, analgesic, anti-inflammatory effects and antioxidant properties [11]. Thus, the aim of this study were to explore the phytochemical constituents, anti-obesity and antioxidant activity of aqueos exteact of *Portulaca oleracea* on dyslipidima in high fat diet rats. We measured markers of oxidative stress and antioxidant state, serum parameters of lipid profile and liver function.

1.- Materials and method

1.1.- Collection and Extraction of Leaves Material

Fresh leaves of the plants were collected in October from a village in Touggourt of Ouargla state, Algeria. The leaves were washed with distilled water and used immediately. The extraction methods described by MAMTA and PARMINDER (2013) [12]. After extraction, the solvents were removed using rotary evaporator, to get gel-like extracts.

1.2.- Phytochemical Screening and HPLC Analysis

The methods of MAMTA and PARMINDER (2013) [12] were used to identify the phytochemicals provides in the extracts: alkaloids, saponins, tannins, steroids, flavonoids,

terpenoids and glycosides. Representation of the components of *P. oleracea* by high performance liquid chromatography indicated that it contains anthocyanins and vitamin C.

1.3.- Determination of Polysaccharides Content

The polysaccharide content was determined according to the phenol–sulfuric acid method. Standard glucose (10 mg) was dissolved with distilled water (100 ml) and then the standard solution was diluted to different concentrations, and finally pure water was added into the diluted solution. 5% phenol (1 ml) was added and mixed up. Then sulfuric acid (5 ml) was added and mixed. The absorbance of the solution was measured at 490 nm with an ultraviolet-visible spectrophotometer, using water as a blank. The standard curve of glucose was: $y = 0.0068 + 0.0169\ x$, $R_2 = 0.9927$ (x represented the concentration of glucose solution, y represented absorbance) [13].

1.4.- Animals and Handling

Twenty four adult male albino rats, weighing 224–238 g, were taken from the animal house of Pasteur institute (Algeria). They were placed in four groups of six rats in each and kept in animal's house of Molecular and cellular biology Department, University of El Oued, Algeria. Standard rat food and tap water were available ad libitum for the duration of the experiments. Animals were adapted for two weeks under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity 64.5 % and room temperature of 22 ± 2 C°. The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution.

1.5.- High fat diet-induced obesity and dyslipidemia in rats

Experimental obesity in Wistar rats was induced by a cafeteria die [14] . along with regular rat chow and water, Peanut (111.11 $g.kg^{-1}$), cookies (111.11 $g.kg^{-1}$), cheese (111.11 g.kg-1), chips (55.55 g.kg-1), salami (55.55 g.kg-1) and chocolate (55.55 g.kg-1) and 50% of the reference group chow diet, before and throughout the experiment.

1.6.- Groups

The high fat diet-induced obesity rats was used. The adult Wistar albino rats were randomly divided into four groups, each containing 6 rats. Control group was given normal diet (served as Normal), untreated group given HFD for 12 weeks (Obese), treated groups were given HFD plus aqueous extracts of *Portulaca oleracea* (400 mg kg^{-1} day^{-1}) administered orally (Obese + P. *oleracea*) and treated groups were given HFD plus Antihypercholesterolemic drug *Atorvastatin* (10 mg kg-1 day-1, p.o) administered orally (Obese +*Ator*). All the groups of animals had free access to water and diet. The gain in body weight was monitored weekly.

1.7.- Blood collection and preparation of tissue samples

At the end of 4 weeks of *P. oleracea* treatment, rats were fasted for 16 hrs, anaesthetized with chloroform by inhalation, rats were decapitated and blood samples were transferred into ice cold centrifuge tubes. The serum was prepared by centrifugation, for 10 min at 3000 revolutions/min and utilized for triglyceride, total cholesterol, HDL concentra-

tions and GOT and GPT activities assays. The blood glucose was measured by glucometer. Absolute liver weight was determined, liver was rapidly excised, weighed and stored at-20°C for oxidative stress parameters analysis.

1.8.- Measurement of biochemical parameters

The activities of glutamate-oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) were determined using commercial kits from Spinreact (Girona, Spain) (refs: GOT-1001161, GPT-1001171). Triglyceride (TG), Total cholesterol (TC), High Density Lipoprotein (HDL) concentrations were also measured using commercial kits (Spinreact) (refs: TG-1001311, TC-1001090, HDL-C-1001096). LDL-cholesterol and VLDL-cholesterol level were calculated indirectly by classic formula.

1.9.- Antioxidants measurement

1.9.1.- Preparation of homogenates

One (1) g of liver was homogenized in 2 ml of buffer solution (phosphate buffer saline, pH=7.4). Homogenates were centrifuged at 10000xg for 15 min at 4°C, and the obtained supernatant was used for the determination of stress oxidative parameters.

1.9.2.- Determination of malondialdehyde (MDA)

MDA was measured according to the method described by SASTRE *et al.* (2000) [15]. Thiobarbituric acid 0.67% (w/v) was added to a liquots of the homogenate previously precipitated with 10% trichloroacetic acid (w/v). Then the mixture was centrifuged, and the supernatant was heated (100°C) for 15 min in a boiling water bath. After cooling, n-butanol was added to neutralize the mixture, and the absorbance was measured at 532 nm. The results were expressed as nmol of MDA/g tissue.

1.9.3.- Determination of reduced glutathione (GSH) level

GSH concentration was measured with the method described by Ellman [16] based on the development of a yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 ml of tissue homogenate was added to 0.2 ml of 0.25% sulphosalylic acid and tubes were centrifuged at 2500 g for 15 min. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml TBS (pH 7.4). Finally, absorbance at 412 nm was recorded. Total GSH content was expressed as nmol GSH/mg prot.

1.9.4.- Assay of Glutathione peroxidase (GSH-Px) activity

Glutathione peroxidase (GSH-Px) catalyzes the reduction of hydroperoxides using GSH as a reductant. Determination of tissue GSH-Px activity was carried out according to the method of FLOHE and GUNZLER (1984) [17]. The reaction mixture contained 0.2 ml of TBS (Tris 50 mM, NaCl 150 mM, pH 7.4); 0.4 ml of GSH (0.1 mM), 0.2 ml of homogenate was added and allowed to equilibrate for 5 min at 25°C. The reaction was initiated by adding 0.2 ml of H2O2 (1.3 mM); reaction was terminated by addition of 1 ml of 1% Trichloroacetic acid (TCA). Tubes were centrifuged at 1500 g for 5 min and the supernatant was collected. To 0.48 ml of resultant supernatant, 2.2 ml of TBS (pH 7.4) and 0.32 ml of DTNB (1.0 mM) were added. After mixing, absorbance was recorded at 412 nm and the specific activity of this enzyme is expressed as µmol GSH/mg protein.

1.10.- Statistical Analysis

The present data were reported as Mean ± SEM. The significance of differences was calculated by using 1-way analysis of variance followed by the Student t test to compare means among the groups. Differences were considered statically significant at $p<0.05$.

2.- Results and discussion

2.1.- Phytochemical Screening polysaccharide content and HPLC analysis.

Phytochemical analysis shown the presence of alkaloids, carbohydrates, tannins and phenolic compounds, flavonoids, triterpenoids, saponins and steroids in the extract (tab. I). The results of the HPLC analysis (fig. 1) show that there is a characteristic peak of ascorbic acid with a retention time of 4.281 and a concentration of 45.03 µg / mg. These antioxidant compounds could have played a major role in scavenging the reactive oxygen species induced by lead acetate [18]

Table I.- Phytochemical composition and polysaccharide analysis of Aqueous extract of *Portulaca oleracea* (+ presence)

Phytochemical	*Portulaca oleracea*
Flavonoids	+++
Tannins	+
Terpenoids	++
Alkaloids	+
Saponins	++
Carbohydrates	+
Steroids	++
Polysaccharide content	0.144 mg/ml

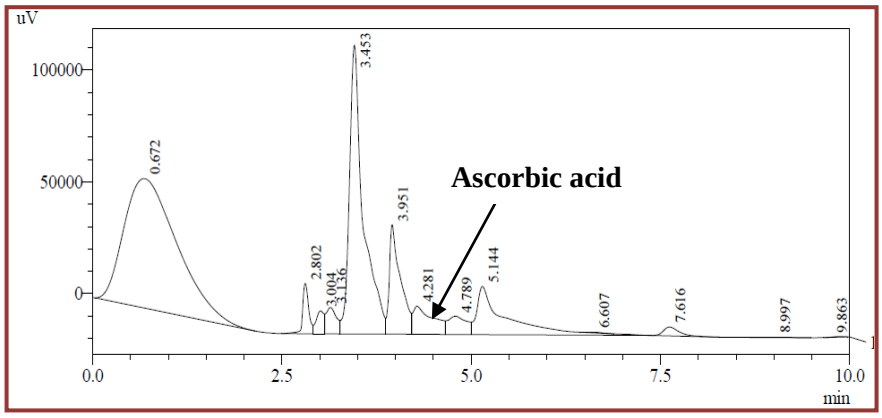

Figure 1.- Ascorbic acid chromatogram

2.2.- Effect of treatments on body weight, food intake and relative lever weight

In this study, after 12 weeks of feeding a HFD to male albino Wistar rats, the body weight was significantly augmented (58.84%) compared with that of the normal diet rats,

indicating that HFD produced obesity (tab. II). The body weight was measured as an indicator of growth and obesity. More consumption of high energy content nutriments such as HFD leads to an rise in the fat mass and fat cell expansion (hypertrophy) without changing food intake, producing the specific pathology of obesity [19]. Aqueous extract of *Portulaca oleracea* was orally administered at 400 mg/kg a period of 4 weeks; in the end aqueous extract of *Portulaca oleracea* reduced the body weight of rats by 39% compared with obese groupe. This result may be due to the decrease of serum total triglyceride, was observed in *P. oleracea* treated obese rats, which caused an amelioration of lipid regulation metabolism [20]. Also, it was observed that *P. oleracea* had no effect on food intake and relative lever weight, except Obese + *Ator* treated group showed significant increase ($p < 0.05$) in relative lever weight and no effect body weight and food intake as compared to Obese animals. (tab. II). This indicates the second effect of this drug on liver function which poses a risk for users.

Table II.- Effect of aqueous extract of *P. oleracea* treatments on body weight, food intake, and weight ratios of liver in experimental rats

Groups	Normal	Obese	Obese + *P.oleracea*	Obese +*Ator*
Initial body weight (g)	229.2±14.5	231.00± 7.02	228.33±7.82	229.17±8.01
Final body weight (g)	246.18±19	366.94± 10.44***	304.26±9.92a	325.59±13.92***
Body weight gain (g/day)	0.566±0.156	4.198±0.114***	2.531±0.077***b	3.214±0.197***
Food intake (g/rat/day)	23.690±0.531	24.923± 0.425***	22.970± 0.590	26.643±0.492**
Weight ratios liver (g/100 g body weight)	2.898±0.144	2.728±0.018	2.723± 0.114	3.008±0.073*b

Data are expressed as Mean ± SEM, (n=6 animals/group), Ator: Atorvastatine
* p<0.05, **p<0.01, ***p<0.001: significantly different from normal group.
a p<0.01,b p<0.001: significantly different from Obese group.

2.3.- Effect of treatments on biochemical parameters.

As seen from table II, blood glucose level and serum lipid concentration was significantly altered which increased the level of glucose ($P < 0.01$), cholesterol, triglycerides ($P < 0.001$), low density lipoprotein (LDL) ($P < 0.01$), and very low density lipoprotein (VLDL) ($P < 0.001$) at the end of the treatment period in HFD animals as compared to normal animals. This suggests that adipose tissue plays a important role in regulating insulin resistance. The increase in the incidence of obesity has been accompanied by a parallel rise in the prevalence of Type 2 Diabetes Mellitus [21]. Accumulation of TGs, TC and LDL-C is one of several risk factors in Coronary Heart Disease (CHD). Also the significant increase of lipid profile in plasma of obese rats might be due to the lack of insulin. Since in normal condition, insulin stimulated the enzyme of lipoprotein lipase and hydrolysis triglycerides [22]. The administration of P. oleracea and Ator to Obese rats restored the changes in the blood and lipid level which decreased the level of glucose (4.85% and 4.70%), cholesterol (10.91% and 9.56%), triglycerides (26.99% and 38.64%), low density lipoprotein (LDL) (16.41% and 7.94%), and very low density lipoprotein (VLDL) (26.99% and 38.64%) and increased the level of high density lipoprotein (HDL) (6.81% and 1.94%) as compared to Obese animals. The result of blood glucose is in line with study of GAO *et al*. (2010), who detected hypoglycemia activity with the aqueous extracts of portulaca oleracea treatment in induced diabetic rats when compared to the non-treated diabetic groupe [23]. Several molecular mechanisms are thought to be involved in the regulation of blood glucose levels after P. oleracea treatment,

bioactive found in portulaca oleracea are, alkaloids, saponins, polyphenols, flavonoids and anthocyanin. These compounds may influence glucose metabolism by some mechanisms, such as inhibition of carbohydrate digestion and glucose absorption in the intestine[21], stimulation of insulin secretion, activation of insulin receptors and glucose uptake and inhibition of hepatic glucose production [24,25]. Our data shown that anthocyanin decrease the levels of serum lipids such as TC and TG in HFD-fed rats, which could be attributed to the inhibition of lipid absorption in the gut. Dietary lipids are absorbed into the blood stream and are then digested to be stored in the liver and adipose tissues in the form of TG [26]. Also P. *oleracea* treatment reduced the level of serum lipid probably by the increase of insulin level in plasma in of obese rats and might prevent the development of Coronary Heart Disease.

Table III.- Effect of aqueous extract of *P. oleracea* treatments on blood parameters in experimental rats

Groups	Normal	Obese	Obese + *P.oleracea*	Obese +*Ator*
Glucose (mg/dl)	89.15±4.71	101.64±3.92**	96.71±3.39^{*a}	96.86± 3.62^{*a}
TG (mg/dl)	79.50±5.30	113.00±8.27***	82.50±6,61^{a}	69.33± 2.95^{*c}
TC (mg/dl)	154.17±1.16	173.80±4.40***	154.83±1,65^{b}	157.17±0.604^{*b}
HDL-C (mg/dl)	52.00±1.81	51.33± 1.76^{NS}	54.83±2.23$^{* b}$	52.33±2.99
LDL-C (mg/dl)	86.27±2.37	99.90±6.53**	83.50±2.45^{b}	91.96±0.897*
VLDL (mg/dl)	15.90±1.06	22.60±1.65***	16.5±1.32^{a}	13.86±0.59^{*c}
GOT (U/l)	138.60 ±3.5	151.2 ±1.4*	49.8±0.8^{***c}	82.60±0.5^{b**}
GPT (U/l)	98.4 ±7.6	144.6 ± 7.7*	56.8± 6.6^{***c}	110. 20±5.4^{a}

Data are expressed as Mean ± SEM, (n=6 animals/group). *Ator*: Atorvastatine
* p<0.05, **p<0.01, ***p<0.001: significantly different from Normal group.
a p<0.05,b p<0.01, c p<0.001: significantly different from Obese group.

2.4.- Effect of treatments on transaminases activities and oxidative stress parameters.

As indicated in figure 2, 3, 4 and table 3 Obese induced a significant decreases in serum reduced glutathione (GSH) level (p<0.001) and GSH-Px activity (p<0.001) and a increase (p<0.001) in serum lipid peroxidation level and SGOT and SGPT activities as compared to Normal rats. This is due to the fact that hepatic cells possess a variety of metabolic activities and contain a host of enzymes. SGPT, SGOT found in higher concentration in cytoplasm and SGPT, principally in mitochondria [27]. Oxidative stress is produced by a relative excess of oxidants, reactive oxygen species [28]. HFD has been identified to increase the liver mitochondrial reactive oxygen species (ROS) production. ROS causes cell injury via the mechanism including lipid peroxidation that leads to tissue damage, especially in the liver [29]. There is a complex interaction between antioxidants and oxidants such as reactive oxygen species, which modulates the generation of oxidative stress [30]. Obesity is also related to a greater risk of developing the liver diseases of different etiologies such as chronic hepatitis C virus (HCV) infection and non-alcoholic fatty liver disease (NAFLD) [31]. In addition, *P. oleracea* treatment led to better correction of these parameters which marked a augmentation of serum GSH (p<0.01) level and GSH-Px (p<0.001) activity and a diminution of serum lipid peroxidation (p<0.01), SGOT and SGPT activities as compared to their Obese group. These observations are symptomatic of antioxidant property of the extracts. Aqueous extract of *Portulaca oleracea* leaves possess flavanoids, saponins, tannins, and phenolic compounds, which are natural antioxidants. polyphenols have also been revealed to provide liver protection against obesity related liver damage [32]. Moreover, the ascorbic acid which proved its existence in our extracts plays an antioxidant role, it makes it possible to limit the mutations of the oxidizing DNA

which protect the liver damage [33].

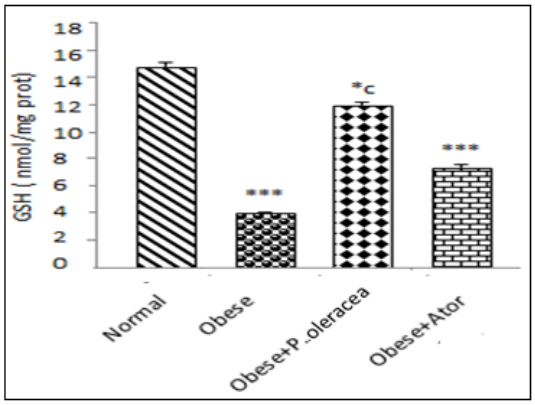

Figure2.- Reduced glutathione (GSH) levels in liver tissue of experimental rats (*p<0.05, ***p<0.001: significantly different from Normal; c p<0.001: significantly different from Obese group)

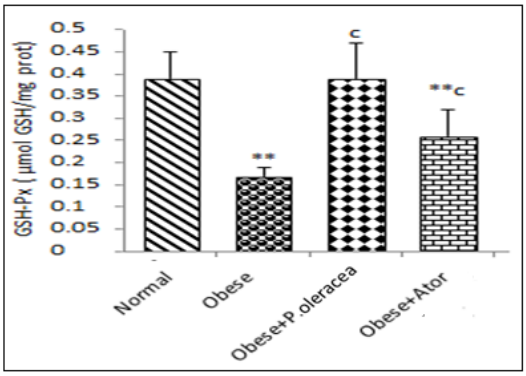

Figure 3.- GSH-Px activity in liver tissue of experimental rats. **p<0.01,: significantly different from Normal group; c p<0.001: significantly different from Obese group.

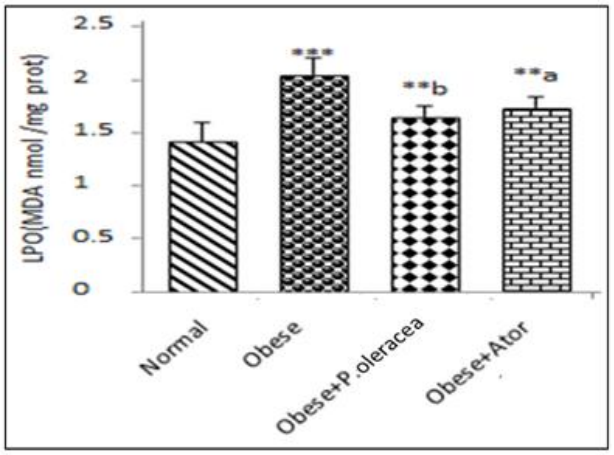

Figure 4.- Lipid peroxidation (LPO) levels in liver tissue of experimental rats (**p<0.01, ***p<0.001: significantly different from Normal group, a p<0.05,b p<0.01: significantly different from Obese group)

Conclusion

In conclusion, these results show that *P. oleracea* has anti-obesity and hepatoprotective effects mediated by antioxidant metabolism and regulation of lipid in obese rats. In addition, *P. oleracea* shows hypoglycemic effect which normalizes the glucose levels in Obese rats.

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