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Phytochemistry analysis and modulatory activity of *Portulaca oleracea* and *Aquilaria malaccensis* extracts against High-fructose and high-fat diet induced immune cells alteration and heart lipid peroxidation in Rats

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Abstract

Our objective of this investigation is to estimate the influence of Portulaca oleracea (P. oleracea) and Aquilaria malaccensis (A. malaccensis) methanol extracts on High-fructose-fat diet (HFFD) induced immune cells alteration and heart lipid peroxidation in Rats. Twenty five Females rats were equally divided into five groups (n=5) as control, HFFD, HFFD+Po, HFFD+Am and HFFD+Po+Amgroups. High fructose-fat diet was addes in diet of rats with (60% fructose and 60% kcal fat) for 70 days. Methanol extracts of P.oleracea (Po) (400mg/kg bw) and A.malaccensis (Am) (200mg/kg bw) were supplemented orally for four weeks. Methanol extracts of plants were prepared and phytochemicals were analyzed by using HPLC methods. Hematological markers and lipid peroxidationin heart were assessed. Results obtained shown that HFFD induction caused a significant increase in White blood cell (P<0.01), Granulocyte (P<0.05), Lymphocyte (P<0.01) Monocyte (P<0.001) count and heart MDA level and no significant effect in Red blood cell and Hemoglobin level compared to the rats given normal diet. Methanol extracts of P. oleracea and A. malaccensis treatment partially correct the parameters studied. Our study indicate that A. malaccensis possesses the ability to control the heart lipid peroxidation and immune cells alteration associated with High fructose-fat diet.

Keywords: *P. oleracea, A. malaccensis,* lipid peroxidation, immune cells, fructose, high fat diet.

Introduction

Nutrition is an essential element of life which is directly related to the health of the population; Therefore, healthy nutrition is a concern of consumers and nutritionists[1]. A food imbalance is a major cause for various pathologies such as diabetes and obesity[2]. Higher levels of fat in foods increase the levels of lipids, such as cholesterol in the blood, increasing the risk of atherosclerosis, obesity, hypertension, diabetes and breast cancer[3]. On the other hand, in an experimental study, food rich in fructose (more than 60% of diet) for 6 months causes the initiation or progression of many diseases such as diabetes and dyslipidemia in rats[4]. In addition, a high calorie diet may cause the risk of liver insulin resistance, a build-up of fat in the liver and skeletal muscle[5]. Oxidative stress is an important factor causing metabolic and physiological alterations and various diseases in the body[6]. Since long time, plants have been used as medicine against several diseases for many diseases; they are still the basis of a system of traditional medicine in different cultures[7]. Among these medicinal plants is *Portulaca oleracea* which is a traditional vegetable very used by indigenous and tribal peoples in many countries. It is known to contain Many active substances are also considered as sources of many dietary supplements[8]. On the other hand *Aquilaria malaccensis* is a plant of the family Thymelaeaceae belongs to the tropical species[9], is one of the main sources of agarwood, which provides clues about their pharmacological properties. Indeed, agar wood is very rich in bioactive substances which favor their use in traditional medicine[10]. In light of these information, our objective for this work is to estimate the benefic role of methanol extracts of *Portulaca oleracea* leaves and the trunk bark of *Aquilaria malaccensis* on high fructose-fat diet induced immune cells disturbance and heart lipid peroxidation in rats.

Materials and methods

Chemicals: Fructose was used as powder provide from (Biomax), (Specialized Food Industry, Algiers, Algeria). All other chemicals are of fine analytical grade.

Plant material: The plants used in this study are the bark trunk *Aquilaria malaccensis* (*A. malaccensis*) were purchased from the local market and the leaves of *Portulaca oleracea* (*P. oleracea*) was harvested in the region of El-Oued "Guemar" in September 2016. The bark of *A.malaccensis* and leave of *P.oleracea* were washed and dried in an airy place and then crushed and stored in powder form until the beginning of the experiment.

Preparation of Methanol extracts: The crude samples (each 3.75g) of each powder mixed with 25ml of methanol-water (70:30) at 25°C for 48h and then filtering with filter paper. After extraction, the solvents were removed under low temperature at 40°C using a rotary evaporator.

Method of Phytochemistry HPLC analysis: The methanol extracts of *Portulaca oleracea* and *Aquilaria malaccensis* were filtered before injection. An HPLC system was used with adetector at λ=280nm for polyphenols and 360nm for flavonoids, the experimental conditions are as follows:

The column used is 150mmx4.6mm with C18 as stationary phase, Mobile phase: acetonitrile and glacial acetic acid (2%, pH=2.6 (30°C). The identification of the peaks of polyphenols and flavonoids was made thanks to the standard achieved by pure components by comparing the retention times.

Animal study: Animals and treatment: Adult female albino rats (aged between 8 to 10 weeks), weighing 200–270g, rats were grouped into five lots of 5 rats in each and reserved in the pet store of our institute. After the adaptation period for two weeks under laboratory conditions (humidity 64.5%, T°=25±2°C and photoperiod (light/dark) 12h/12 h, Put water and food at the disposal of rats freely. Food is richon fructose (60% of fructose of diet)[11] and fat diet (60% fat)[12] (HFFD) adlibitum for 70 days. The five groups of rats are distributed as follows:

Group 1 (control group): animals were given normal diet served as control.
Group 2 (HFFD): Rats were given HFFD diet.
Group 3 (HFFD+Po): Rats were given HFFD diet plus methanol extracts of *P.oleracea* (400 mg kg-1 d-1) administered orally.
Group 4 (HFFD+Am): Rats were given HFFD diet plus methanol extracts of *A.malaccensis* ($200mg.kg^{-1}.d^{-1}$) administered orally.
Group 5 (HFFD+Po+Am): Rats were given HFFD diet plus both methanol extracts of *P.oleracea* and *A.malaccensis* administered orally.

The duration of different treatment is 30 days. Body weight was recorded regularly.

Collection of biological materials: After 30 days of treatment, the rats are sacrificed after at least 16 hours of fasting by decapitation under inhalation chloroform anesthetized. The sample of blood is carried in tubes of EDTA for hematological studies (hematological analysis (FNS) is performed by the hematology auto analyzer (Sysmex)). Heart was quickly removed, absolute heart weight was determined than stored at -20°C for lipid peroxidation analysis.

Lipid peroxidation measurement: Preparation of homogenates: Mixt 1g of heart tissue with 9ml of buffer solution (KH_2PO_4, pH=7.4). After grinding of the mixture, the homogenate is obtained by used centrifugation at 9000xg, 15min at 4°C than the homogenate samples are stored at -20°C for MDA assay.

Estimation of Malondialdehyde (MDA) levels: Malondialdhyde analysis is based on the thiobarbituric acid (TBA) technique following the method of Sastre et al.[13] by measuring the absorbance of TBA-MDA complex at 530nm. The results presented by μMol/mg protein.

Protein determination: Estimation of the tissue protein is based on the Braford method[14]. The standard curve is made by the BSA.

Statistical Analysis: The present results were reported as Mean and standard error of mean. The comparison between the means is made by Student t test. Statistically significance threshold of results using α=p<0.05. *p<0.05, **p<0.01, ***p<0.001: comparison with control group, a p<0.05, bp<0.01, cp<0.001: comparison with HFFD group.

Results and discussion

Polyphenol HPLC analyzes: Chromatogram of HPLC analysis (Figure-1) show that the methanol extract of *P. oleracia* and *A. malaccencis* are rich in polyphenol in different quantities and that Apigenin, Epicatechin and Naringenin has the major substance among these constituents of *P.oleracia* and *A.malaccensis*.

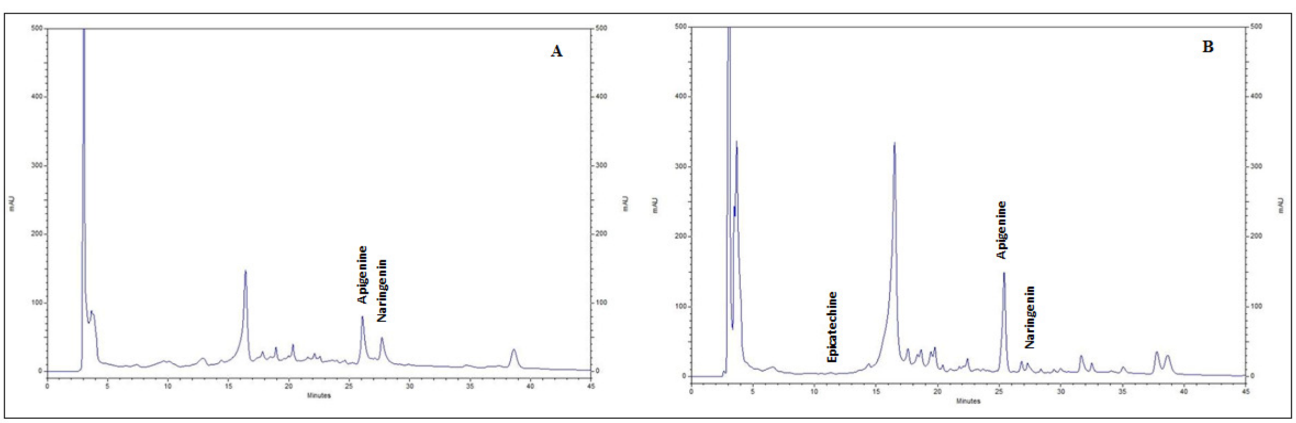

Figure-1: Chromatogram of polyphenol for the methanol extract of *P.oleracia* (A) and *A.malaccensis* (B).

Flavonoid HPLC analyzes: The results of the chromatographic analysis by HPLC show that the methanol extract of *P.oleracia* and *A.malaccencis* are rich in flavoinoids in different quantities and that Quercetin, Kampferol and Rutinhas the majority substance for *P.oleracia* and *A.malaccensis* (Figure-2).

Body weight and relative heart weight: As seen in Table-1, results show that compared to the control, the weight gain was increased ($p<0.01$) in group of rats under HFFD. However, methanol extract treatment causes a significant decrease ($p<0.05$, $p<0.001$ and $p<0.01$) of weight gain in HFFD+Po, HFFD+Am and HFFD+Am+Po groups respectively compared to the HFFD group. The results obtained show a no significant variation in the relative weight of heart between HFFD and control groups (Table-1).

Hematological markers: As seen from Table-2, results showed a significant increase of White blood cell (WBC) ($P<0.01$), Lymphocyte (LYM) ($P<0.01$), Granulocyte (GRN) ($P<0.05$) and Monocyte (MON) ($P<0.001$) count and no significant change of RBC and Hemoglobin (HGB) level in HFFD animals as compared to normal animals. Moreover, the results obtained show that there is a significant improvement in the WBC, LYM, GRN and MON count in the rats treated with extract of *A. malaccensis* (Am) and Po+Am compared to HFFD group. While, only *P. oleracea* (Po) treatment significantly decreases the WBC count compared to the HFFD group.

Level of lipid peroxidation marker: The obtained results show that compared to control, the lipid peroxidation in heart was increased ($p<0.05$) in HFFD group. While treatment with *A.malaccensis* (Am) or Por+Aq leads to reduction ($p<0.05$) in heart MDA levels when compared to the HFFD group. But no effect of *P. oleracea* (Po) extract treatment on heart lipid peroxidation compared to HFFD group (Figure-3).

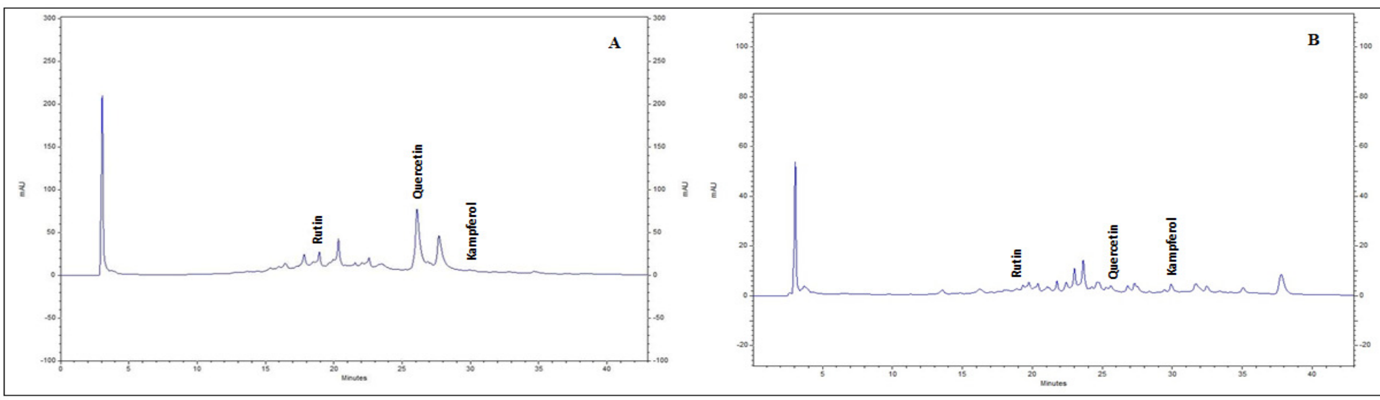

Figure-2: Chromatogram of flavonoids for the methanol extract of *P. oleracia* (A) and *A. malaccensis* (B).

Table-1: Body weight and relative heart weight of experimental groups.

Groups	Control	HFFD	HFFD + Po	HFFD +Am	HFFD +Am+Po
Initial body weight (g)	203.3±2.3	268.5±1.96	239.25±6.87	241±4.04	230.75±5.06
Body weight gain (g/day)	0.53±0.055	1.05±0.036**	0.26±0.25^{a}	0.14±0.06^{c}	0. 8±0.06^{b}
Relative heart Weight (g/100g b.w)	2.8±0.05	2.7±0.16	2.9±0.20	2.9±0.09	2.8±0.16

Table-2: Meanhematological markers in experimental groups.

Groups	Control	HFFD	HFFD + Po	HFFD +Am	HFFD +Am+Po
RBC (10^6/µL)	7.02±0.20	6.86±0.22	6.95±0.15	7.22±0.23	7.13±0.11
HGB (g/dL)	13.42±0.36	13.03±0.37	13.37±0.33	13.85±0.45	13.55±0.42
WBC (10^3/uL)	3.62±0.14	4.9±0.12**	4.37±0.18*	4.0±0.11^{a}	3.27±0.27^{a}
LYM (U/l)	3.08±0.12	4.03±0.09**	3.98±0.05^{*a}	3.68±0.1^{a}	2.86±0.12^{b}
MON (10^3/µl)	0.28±0.01	0.68±0.01***	0.57±0.06*	0.45±0.05*^{a}	0.35±0.05^{b}
GRN (10^3/µl)	0.34±0.04	0.53±0.02**	0.46±0.01	0.39±0.06^{a}	0.31±0.01^{a}

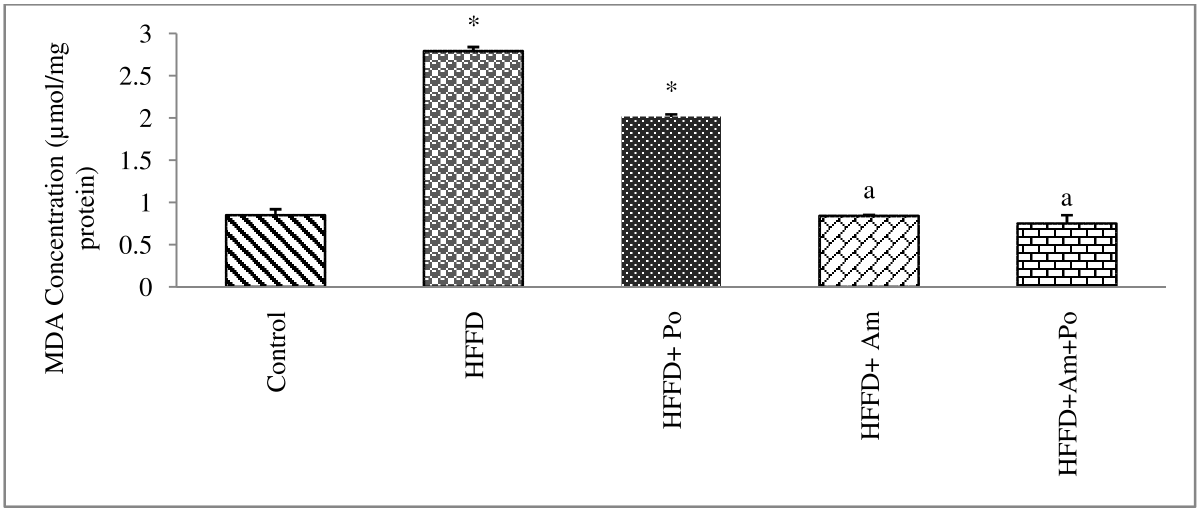

Figure-3: Mean MDA level in heart tissue of control and experimental rats.

Discussion: The results of chromatography HPLC analysis reveal that the methanol extract of dry leaves of *Portulaca oleracea* and the trunk bark of *Aquilaria malaccensis* contain several bioactive compounds, including Apigenin, Epicatechin, Naringenin Quercetin, Kampferol and Rutin. Phytochemicals in the diet can exert on different targets that can relieve multiple pathological processes, including oxidative damage, epigenetic alterations, chronic inflammation, active stimulators, inhibitors and growth terminators and prevention of various diseases associated with oxidative stress[15,16]. In our study, results show that HFFD food causes a rise inweight gain resulting an obese phenotype of rats. More consumption of high energy content nutriments such as fructose and HFD leads to an rise in the fat mass and fat cell expansion (hypertrophy)without changing food intake, producing the specific pathology of obesity[17]. Also high proportion of lipids in food can increase palatability and cause hyperphagia of animals leads to rapid weight gain[18]. Treatment of HFFD rats with the methanol extract of *P.oleracea* and / or *A.malaccensis* induces a decrease in body weight gain. The anti-obesity properties of plants can be exerted according to different modes of action: by direct effect on food intake by suppressing appetite and inducing the feeling of satiety, a reduction of lipid absorption, a reduction of energy consumption, increase in energy consumption, slow development of pre-adipocytes, decrease energy intake from the gastrointestinal tract[19,20]. Our results reveal that the administration of HFFD in rats causes an increase in white blood cells, granulocytes, as well as lymphocytes and monocytes compared to control, these results is of support with study of Gomez-Smith et al.[21] who used high fat diet model alone and the Pektas et al. study, which showed that levels of pro-inflammatory factors increased by high fructose content[22]. A high fructose diet may induce inflammation, as fructose in combination with an increase in monocyte-macrophage infiltration[23]. In addition, fructose exerts a pro-oxidative and pro-inflammatory effect, since it activates a pro-oxidant enzyme NADPH-oxidase, responsible for the release of superoxide anion[24]. White blood cell and lymphocyte count analysis is a very important diagnostic test to detect the presence of inflammation and other diseases[25]. A complete blood count also is needed to a control of the meaning of the changes of the immune cells in the body, such as, the elevation of the granulocyte presents a risk of attacking a heart failure in the human, whereas a decreased risk has been controlled by the rise of monocytesnumber[26]. According to our results, treatment with *Portulaca oleracea* exerts an anti-inflammatory effect in HFFD rats, which is similar with many studies, Lee et al. shows by an experiment in mice that the *P. oleracea* suppresses inflammation at the vascular level[27], Xiaohang et al reveals that *P.oleracea* reduces expression of cytokine suffer from ulcerative colitis[28]. Many nutritional and pharmacological agents, including polyphenols and flavonoids have been studied to determine their anti-inflammatory effects[29]. Many studies have shown that flavonoids exhibit their pharmacological activities, including anti-inflammatory, through the inhibition of important regulatory enzymes[30]. Apigenin, a polyphenol, inhibits the proliferation and apoptosis of monocytes and lymphocytes during leukemia and inhibits the platelet by blocking TxA (2) (TP) receptors[31]. The treatment with *Aquilaria malaccensis* leading to a reduction of inflammatory signs in rats, probably because of the richness of the plant in different secondary metabolites such as polyphenols and flavonoids. In addition agarwood contains bioactive substances such as 2-(2-phenylethyl) chromones and eudesmol, guaiene[32], these compounds have identified by their anti-inflammatory and immunomodulatory activities by reducing the toxicity of cytokines[33]. Our results show that the level of cardiac MDA was higher in HFFD -fed rats than the control. High fructose-fat diet has been identified to increase oxidative stress in tissues. Free radical causes cell injury via the mechanism including lipid peroxidation that leads to tissue damage[34]. Excessive ROS production attacks local cell organelles, including membrane lipids, resulting in lipid peroxidation[35]. The reduction of lipid peroxidation rate after the treatment of *A.malacenssis*, may be due to antioxidant substances presented in the extract, HPLC analysis of crude extracts of *A. malacensisis* reveal the presence of Apigenin, Epicatechin, Naringenin Quercetin, Kampferol and Rutin. Some these phytochemicals have antioxidant activity where they offer protection against damage and the risk of developing metabolic diseases[36]. The potent molecule such as flavonoids was present in the bark and also the plant has an important antioxidant value[37]and also has a significant activity of scanning of free radicals[38].

Conclusion

In conclusion, this study clearly concluded that the Methanol extract of bark *A.malaccensis* and leave *P.oleracea* possess the ability to control the immune cells associated with HFFD, and protective activity on heart cells, which in turn improve cardiovascular systems.

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