

European Journal of Medicinal Plants

23(4): 1-10, 2018; Article no.EJMP.40946 ISSN: 2231-0894, NLM ID: 101583475

Effect of *Opuntia ficus indica* on Antioxidant Activity and Lipid Profile of Experimental Rats Ingested Thermally Oxidized Oil

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Authors' contributions

This work was carried out in collaboration between all authors. Author YHP designed the study and wrote the protocol. Authors SH, AM, NZ and KB managed the analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/40946

Editor(s)

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Complete Peer review History: http://www.sciencedomain.org/review-history/24675

Original Research Article

Received 14th February 2018 Accepted 23th April 2018 Published 18th May 2018

ABSTRACT

Opuntia ficus indica largely presents in Algerian rural environment, is frequently used by the local population for its therapeutic virtues. This particular study was carried out on Wistar albino rats, which were divided into four individual groups namely I, II, III and IV. The rats in Group I served as the control, received a distilled water, Group II was received thermoxidized vegetable oil, while Group III and IV were received thermoxidized oil with 100 and 500 mg/kg body weight of Opuntia ficus indica aqueous extract added in each respectively. At the end of 22 days administration, blood samples were collected for the analysis of lipid profile. The result showed that TG was significantly increased (P<0.05) in all the test groups when compared with the control, The HDL was significantly (P<0.05) increased in all the test samples except GroupII, while LDL followed a reverse trend. The phytochemical studies showed the presence of phytoconstituents like alkaloids, flavonoids and phenols.

Keywords: Opuntia ficus indica; hypercholesterolemia; thermally oxidized oil; wistar rats.

1. INTRODUCTION

Cholesterol is a lipid (grease). It is Produced by the body but can also be taken in from food; it's actually plays a very important role in the functioning of the body. Cholesterol is present in the membrane (external layer) of all the cells of the body.

Cholesterol is transported in the blood by molecules called lipoproteins. They are divided into three types: Low-density lipoprotein (LDL) (bad cholesterol) would be responsible for the arterial diseases. The LDL transports the cholesterol of the liver towards the cells and can involve a harmful accumulation if the quantity is higher than what the cells can use [1]. Highdensity lipoprotein (HDL) (good cholesterol) would be responsible for the prevention of the arterial diseases. The HDL removes the cholesterol of the cells to bring it towards the liver where it is destroyed and eliminated from the body in the form of waste. The triglycerides, produced by the liver, are in the dairy products, the meat and oils of cooking [2].

Much of the fat consumed in our diet has been exposed to heat during processing and in the preparation of food during cooking. In deepfrying, oil is heated above 150°C for a long period. This heating process causes changes in the properties of the oil by chemical reactions of oxidation, hydrolysis and polymerization. When frying oil is heated, hydroperoxides and aldehydes are mainly formed and are absorbed into the fried foods. Thus, these products enter the systemic circulation. Malondialdehyde (MDA) is usually measured as a marker for lipid peroxidation level. Thiobarbituric acid (TBA) assay is the most common method to be used to measure MDA. However, it is more appropriate to express the results as thiobarbituric acid reactive substances (TBARS) rather than MDA because other products may also form during the assay [3]

The common practice of repeatedly using the oil for frying may generate free radicals that are harmful to our health. Such practice appears to cut the cost of cooking without considering its effects on health. Several studies had demonstrated the adverse effects of oxidized dietary fats on human and experimental animals. [3]

The *Opuntia* belongs to the family of *Cactacées* which is known by the presence of the spines on the level of the nodes (areolas) and to a subfamily of *Opuntiodées* which is characterized by the presence of glochides. It is introduced into the Mediterranean region towards the end of the 15th century and in North Africa towards the end of 16th [4].

The cactus is a xerophytic plant which produces edible fruits and fodder for the cattle. Its rackets are rich in water and nutritive elements.

Besides the valorization of the rackets in livestock feed and young cladodes in the diet of man, the mucilage of the rackets is used in the manufacturing of the champions, the softeners of the hair, the dermic creams and hydrating milk [5]. This mucilage also makes it possible to reduce the cholesterol level in blood. The dried powder of the rackets or powder of nopal also affects control of sugar and cholesterol in the blood. It has also an effect antiglycemic, the pectin of the powder has been shown to decrease plasma Cholesterol [5,6].

The aim of this study it's to determine the effect of aqueous extract of *Opuntia ficus indica* to reduce a level of cholesterol produced by thermally oxidized vegetable oil.

2. MATERIALS AND METHODS

2.1 Plant Material

Opuntia ficus indica cladodes used in this study were collected from the el-Khroub region of Constantine (Algeria) in 2015. The annual average temperatures are in the order of 15.15°C. The maximum of temperatures reaches 33.58°C in July, whereas the minimum reaches 2.73°C in January. The pluviometry average is of 600 mm.

2.1.1 Preparation of the extract

Aqueous extract: After the harvest of the plant material, each sample was cut into thin slices and dried in a stove at a temperature of 40°C for 72 hours. It ground using a grinder (type Gulatti MFC) rotated at the speed of 1000 rpm and equipped with a sieve, a square mesh sizes from 0.85 mm.

Fifty grams of the powder were introduced into an Erlenmeyer flask and macerated in 500 ml of distilled water with mechanical stirring for 24 hours at ambient temperature. Then it was filtered through Whatman filter paper, the filtrate (Aqueous extract, EAq) obtained was evaporated using rotary evaporator (BÜCHI) [1]. The extract was transferred to a closed container and stored at -4°C until the time of use.

Organic solvents extraction and fractionation: The extraction method we used is the successive maceration with three solvents of increasing polarity (petroleum ether, chloroform and methanol) [7].

250 g of powder was extracted with 750 ml of petroleum ether and placed under mechanical stirring for 24 hours at room temperature. After filtration, the residue was taken up in 750 ml of chloroform and left stirring for 24 hours; the residue is again extracted with 750 ml of methanol for 24 hours under the same conditions. The process was repeated thrice, and the extracts were combined. The solvent was evaporated on the rotary evaporator (BUCHI) to obtain a different extract (EEp, EChI, EMet).

The methanolic extract was then suspended in distilled water in a separatory funnel and partitioned successively with ethyl acetate, and n-butanol to obtain fractions in these solvents. This process left residual aqueous fraction at the end. The solvents were removed on a rotary evaporator at low pressure to get dried fractions (EAc, EBut).

2.2 Preparation of Thermoxidized Oil

The vegetable oil used was heated five times [8]. The temperature of the heated oil reached about 180°C for 15 minutes. Between each heated the oil was cooled for 5 hours [9].

2.3 Animals

Young albino Wistar rats of either sex aged 4-5 weeks, average weight 150-250 g were used for the experiment. The rats were purchased from the Pasteur Institute in Algiers. They were kept in standard environmental condition (at 24±1°C temperature and 55-65% relative humidity and 12-hour light/12 hour dark cycle) for one week for acclimation after their purchase with free access to food and water.

2.4 Methods

2.4.1 Phytochemical screening

The extract was tested for the presence of bioactive compounds by using following standard methods [10-13].

Test for reducing sugars (Fehling's test): Their detection involves treating 1 ml of the extract with 2 ml of distilled water, 20 drops of Fehling's solution and then heated. The appearance of yellow and then brick red precipitate indicates the presence of reducing sugars [13].

Test for Triterpenoids: To extract solution, ten drops of acetic anhydride was added and mixed well. To this, concentrated sulphuric acid was added from the sides of the test tube. The appearance of greenish blue colour indicates the presence of triterpenoids [12].

Test for flavonoids: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on the addition of dilute acid, indicates the presence of flavonoids [11].

Test for saponins: The extract solution was mixed with 5 ml of water and vigorously shaken. The formation of the stable foams indicated the presence of saponins [11,12].

Test for tannins: The diluted ferric chloride solution was added to extract in a test tube and observation made. The appearance of dark green colour or blue-green indicates the presence of tannins [11].

Test for alkaloids: Extract was dissolved in dilute Hydrochloric acid and filtered. The filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids [11].

Test for steroids: Extract was mixed with chloroform and concentrated sulphuric acid was added. A red colour produced in the lower chloroform layer indicated the presence of steroids [12].

2.4.2 Test of the lipidic peroxidation by the method of the TBARS

The method of Choi et al. 2002, [14] using induction of lipid peroxidation by ascorbic acid /

ferrous sulfate (Fenton reaction) has been adapted for this test. 600 µL of different concentrations (100, 250, 500, 750 and 1000 µg/ml) from extracts of O.f.indica (EEp, EChl, Emet, EAc, EBut and EAq), was respectively added with 300 µL of buffer solution Tris-HCl (pH 7.5; 20 mM), 500 µL of linoleic acid (20 mM) and 100 µL of ferrous sulphate (4 mM). The peroxidation begins after addition of 100 µL of ascorbic acid (5 mM). The resulting reaction mixture was incubated in a water bath at 37°C for 60 minutes. After this step, 2 ml of TCA (10%) were added to all tubes. Then, one mL of aliquot collected in each of the reaction mixtures prepared previously is added one mL of TBA (1%). The reaction mixtures obtained are placed in boiling water bath at 95°C for 20 minutes.

The Gallic acid (AGA) is used as a reference molecule. A white test is carried out by replacing the extract with distilled water. [15]

Absorbance is read with the spectrophotometer at 532 Nm and percentage of inhibition of linoleic acid is determined using the following equation:

Inhibition (%) = (1- (DO test / blank DO))x100

2.4.3 Test of the hypocholesterolemic activity of the aqueous extract of Opuntia ficus indica

The rats are placed in cages where each cage gathers sixr rats. They have free access to water and food. The rats were maintained at an ambient temperature of 24°C and a photoperiod of 12-hour light/12 hour dark. They were treated by the principle and guideline set out in the handbook on the care and use of experimental animals [16].

Animal groups [15]

- Group I (6 rats) healthy control: the subjects received daily by gastric gavage 2ml of distilled water.
- Group II (6 rats) control intoxicated: the subjects received each day by gastric gavage 2 ml of oxidized oil + 0.5 ml of physiological water by intraperitoneal injection.
- Group III (6 rats): the rats received each day by gastric gavage 2 ml of oxidized oil + 0.5 ml of the aqueous extract of Opuntia

- ficus indica (100 mg/BW) by intraperitoneal injection.
- **Group IV (6 rats):** the rats received each day by gastric gavage 2 ml of oxidized oil + 0.5ml of the aqueous extract of *Opuntia ficus indica* of (500 mg/BW) by intraperitoneal injection.

> Collection of Blood and Serum Samples

At the end of the experiment (Day 22), the blood sample was collected in sterile tubes, centrifuged at 3000 rpm for 10 minutes. The serum was recovered and used for the biochemical assays of TG, HDL and LDL cholesterol.

The select blood parameters were performed in the biochemical laboratory of Constantine.

> Body Weight and Organs Measurement

The animals were sacrificed after anaesthetizing with chloroform, and the various organs were collected and weighed: liver, spleen, lung, heart and kidneys. The relative weight values of organs were calculated by this equation [(organ weight/rat weight) x 100].

The body weight measurement is performed on the rats, regularly, every week just before the blood samples.

2.4.4 *In vivo* evaluation of lipid peroxidation

The lipid peroxidation in the liver was evaluated by the malondialdehyde (MDA) assay according to the method of Uchiyama and Mihara [17]. One gram of liver was added to 5 ml of KCl solution (1.15%) and then homogenized. To 0.5 ml of the homogenate, 3 ml of phosphoric acid (1%) and 1 ml of thiobarbituric acid (TBA: 0.67%) were added. The mixture was heated at 100°C for 45 minutes, cooled and then added 4 ml of n-butanol. After centrifugation for 15 minutes at 3000 rpm, the absorbance was determined on the supernatant at 532 nm. [18].

The concentration of MDA is deduced from a standard range established under the same conditions with 1, 1, 3, 3-tetraetoxypropane which gives the MDA after its hydrolysis in solution. The results of the assay were expressed in nmol/gram of liver (Fig. 1).

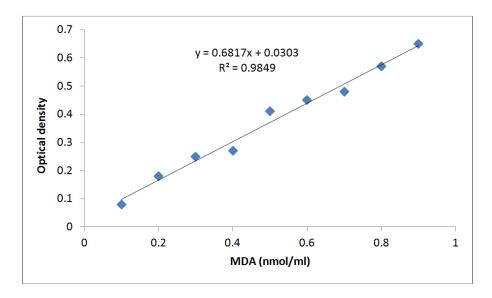


Fig. 1. MDA calibration curve with TBA reagent

2.5 Statistical Analysis

The results of the statistical analysis for the animal experiment were expressed as mean \pm SEM and were evaluated by ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group. The p<0.05, was considered to be statistically significant.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

The results of preliminary phytochemical screening carried out on the *Opuntia ficus indica* cladodes powder were shown in Table 1. From these results, Flavonoids, steroids, Tannins, saponins and reducing sugars were detected. However, they were devoid of alkaloids and Triterpenoids.

3.2 Lipid Peroxidation Test Using the TBARS Method

Fig. 2 shows the percentage of inhibition of the peroxidation of linoleic acid in the presence of $% \left(1\right) =\left(1\right) \left(1\right)$

various extracts of *Opuntia ficus indica* and gallic acid (AGA). The absorbances indicating a strong antioxidant activity compared to control.

At a concentration of 100 μ g/ml, the ethyl acetate, butanol and aqueous extracts exert inhibitory effects of the peroxidation of 94.7%; 99.4% and 90.3 respectively. These values are statistically similar to that of Gallic acid (AGA) which exerts an inhibitory effect of 96.1% at the same concentration; they have the strongest antiradical activities Also, EEp, EChl and EMet present a good antioxidant activity but weak compared to those extracts (Fig. 2).

Indeed, the analysis of the variance (ANOVA) showed that the inhibition percentages of these extracts were statistically significantly different from that of the control that represents gallic acid.

Also, these extracts comprise, within them, a phenolic compound whose content is in adequacy with the antiradical activity translated here by the lipid peroxidation test by the TBARS method.

Table 1. Results of phytochemicals tests of the aqueous extract of *Opuntia ficus indica* cladodes

Tests	Reducing sugars	Triterpenoids	Flavonoids	saponins	Tannins	alkaloids	steroids
Aqueous extract	+++	-	+++	+	+	-	+++

+++ Very positive Reaction; + Positive Reaction; - Negative Reaction

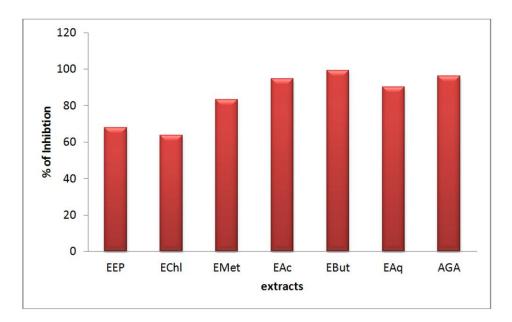


Fig. 2. The percentage of inhibition of the peroxidation of linoleic acid in the presence of various extracts of *Opuntia ficus indica* and gallic acid

According to Hsu et al. [19], the phenolic compounds are widely distributed in plant tissues, among which are numerous antiradical and antioxidant molecules. Moreover, Alain dit P. B et al. [20], Duh et al. [21] and N'guessan et al. [22] showed a correlation between total phenol levels and antiradical activity.

According to Chen and Ho [23], the functional groups present in phenolic compounds can easily give up an electron or a proton to neutralize the free radicals. The strong antioxidant activity of the three extracts would be thus related to their high total phenol content.

Our results are in agreement with the work of Adedapo A.A., et al. [24]. According to the authors, plants which have a good antioxidant activity contain high levels of phenolic groups.

Phytochemical screening shows that EAC, EBut and EAq extracts also contain flavonoids, metabolites possess good antioxidant activity, polyterpenes, saponins and alkaloids. Their strong antioxidant activity would contribute to their various therapeutic activities [10].

The work of Sandhar et al. In 2011 [25] shows that flavonoids inhibit lipid peroxidation at an early stage by the scavenger activity of peroxide radicals as they can interrupt a chain of radical reactions by the property of hydrogen donation.

These data reveal that the potent inhibitory activity of lipid peroxidation of the three extracts from *Opuntia ficus indica* is due to their excellent anti-radical activity proven by the other tests. This ability to modify lipid peroxidation induced by free radicals is related not only to the structural characteristics of antioxidants but also to their ability to interact with and penetrate in the lipid bilayers.

It has been shown that the structure and lipophilicity of polyphenols are factors that determine the antioxidative property, probably affecting the depth of incorporation of these compounds into the lipidic phase of the membrane [26].

3.3 Hypocholesterolemic Activity of the Aqueous Extract of *Opuntia ficus indica*

Lipid Profile

The TG concentration showed a significant increase (P<0.05) in all the test groups when compared with the control (Group I). The HDL-C reveals a significant (P<0.05) elevation in all the experimental groups except group II which showed a significant (P<0.05) decrease when compared with the control. The results of low-density lipoprotein-cholesterol (LDL-C) showed a significant (P<0.05) reduction in LDL- in all the

test groups except group II which showed a significant (P<0.05) increase when compared with the control, (Table 2).

The assessment of the lipid profile showed a significant increase in TG of all the test groups. However, there was a significant increase in the HDL-C of all the test groups but a reverse in the case of GroupII and a significant decrease in LDL-C in all the test groups but a reverse effect by Group II.

A rise in TG has been associated with Coronary heart disease CHD [27,28]. The increase in TG, in this case, could cause the liver to form other types of lipids particularly the phospholipids [27, 28]. However, the obvious significant decrease in the HDL-C with a concomitant significant increase in the LDL-C level in GroupII indicates a significant shift towards formation of bad cholesterol (LDL-C) but the addition the aqueous extract of Opuntia ficus indica was seen to reverse this shift in Group III and Group IV thereby stabilizing the production of good cholesterol (HDL-C). Clinically, increased HDL is beneficial to health since it reduces the risk of coronary heart disease (CHD). Thermoxidized oils contain substances such as core aldehydes and 9-oxononanoic acid which are injurious to the body by inducing lipid peroxidation and altering hepatic metabolism through the production of free radicals causing an increased level of LDL-C [27,28]. LDL-C is known to be the primary marker for a number of degenerative diseases, particularly arteriosclerosis [29] The presence of phytochemicals in the Opuntia ficus indica such as reducing sugars, flavonoids and phenolic acids may have mopped up the free radicals produced by Thermoxidized oil [29] restoring an improved HDL-C level as seen in Group III and Group IV. Also, Eddouks et al. [30] have reported that Opuntia ficus indica contains

phenolic substances which lower blood cholesterol in the rat.

Changes in Body Weight

At day 22, the gain of body weight in hypercholesteremic (Group II), hypercholesteremic rats treated with both doses (100mg and 500mg) of the aqueous extract of *Opuntia ficus indica* was different to that obtained in the untreated group. Indeed the values represent 0.015; 0.016; 0.017; and 0.029 Kg respectively (Table 3).

Absolute and relative values of organ weights:

The weight of the liver, heart, kidneys, spleen and lungs shows no significant difference in the treated group compared to the control group, whereas it present a significant difference between the control intoxicated group and the control group (Table 4).

The relative weight values of organs were not influenced by the hypercholesterolemic diet supplemented with the aqueous extract of *Opuntia ficus indica*.

The weight of the liver, heart, kidneys, spleen and lungs shows no significant difference in the treated group compared to the control group, whereas it presents a significant difference between the control intoxicated group and the control group (Table 5).

The relative weight values of organs were not influenced by the hypercholesterolemic diet supplemented with the aqueous extract of *Opuntia ficus indica*.

Table 2. Effect of *Opuntia ficus indica* on TG, HDL and LDL cholesterol of rats treated with oxidized oil

Groups	Group I	Group II	Group III	Group IV
Triglyceride (TG), g/l	0.58 ± 0.183	0.73 ± 0,169	0.625 ± 0.254	0.615 ± 0.134
Cholesterol-HDL, g/l	0.24 ± 0.042	$0.205 \pm 0,007$	$0.25 \pm 0,152$	0.27 ± 0.034
Cholesterol-LDL, g/l	0.33 ± 0.084	0.41 ± 0.007	0.31 ± 0.046	0.22 ± 0.036

Table 3. Body weight variation

	Group I	Group II	Group III	Group IV
Initial weight (KG)	0.117 ± 0.012	0.118 ± 0.007	0.128 ± 0.009	0.107 ± 0.005
Final weight (KG)	0.146 ± 0.020	0.133 ± 0.022	0.144 ± 0.01	0.124 ± 0.016
Weight gains (KG) after 22 days	0.029	0.015	0.016	0.017

Table 4. Organ weights

	Group I	Group II	Group III	Group IV
Organ weights (g)				
Liver	6.13±0.509	5.506±0.794	5.982±0.753	5.995±2.001
Heart	0.595±0.091	0.47±0.043	0.582±0.040	0.555±0.091
Kidneys	0.92±0.07	0.846±0.025	0.927±0.136	0.89±0.197
Spleen	0.585±0.049	0.456±0.086	0.565±0.129	0.515±0.261
Lung	1.31±0.212	2.363±0.899	1.23±0.204	1.305±0.233
Relative weight				
Liver	4.198±0.481	4.440±0.745	4.154±0.716	4.507±1.046
Heart	0.407±0.071	0.379±0.012	0.404±0.011	0.417±0.104
Kidneys	0.630±0.03	0.682±0.011	0.643±0.101	0.669±0.127
Spleen	0.400±0.023	0.367±0.047	0.392±0.094	0.387±0.241
Lung	0.897±0.178	1.905±0.872	0.854±0.176	0.981±0.198

Table 5. Effect of the aqueous extract of *Opuntia ficus indica* on the production of MDA in liver cells

	Group I	Group II	Group III	Group IV
MDA (nmol)	2.0442±0.005	6.5232±0.0262	2.724±0.001	1.9906±0.001

3.4 *In vivo* Evaluation of Lipid Peroxidation

We noted a rise of very highly significant (p< 0.001) of the MDA in the rats receiving oil oxidized and untreated (6.5232±0.0262 nmol) compared to the normal control group (2.0442±0.005 nmol). Also, no significant variation of the MDA is noted in the rats receiving oil oxidized and pretreated by the aqueous extract with at doses of 100 and 500 mg (their respective values 2.724±0.001 nmol and 1.9906±0.001 nmol do not have any significant difference with that obtained with the normal control). This result probably explains the protection of the animals by the bioactive substances existing in the aqueous extract of *Opuntia ficus indica* against the oxidative stress.

According to the work of Rasekh et al. [31], the aqueous extract of *T. policema*n causes a reduction in total cholesterol levels in rats. These authors attribute this decrease to the presence of some polyphenols and flavonoids endowed with anti-hyperlipidemic properties.

Mary Sujin et al., [32] explained the reduction in the rate of cholesterol in plasma by a problem of formation of micelles and biliary acids in the digestive tract what interferes with absorption of cholesterol, and in this case, the cholestérole is excreted.

Thus the reduction in the cholesterol noticed in our results requires more research and analyses to explain its causes.

4. CONCLUSION

The common practice of repeatedly using the oil for frying may generate free radicals that are harmful to our health. Such practice appears to cut the cost of cooking without considering its effects on health. The results of this study demonstrated the adverse effects of oxidized oil on experimental animals. *Opuntia ficus indica* aqueous extract has améliorative effects probably due to its numerous bioactive constituents and their antioxidative activities.

CONSENT

It is not applicable.

ETHICAL STATEMENT

This study was carried out in strict accordance with the recommendations in the Guide for the Care and use of Laboratory Animals of the National Institutes of Public Health of Algeria (INSP). The protocol was approved by the committee on the Ethics of Animal Experiments of Mentouri Brothers University Constantine1.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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