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# *In vivo* anti-inflammatory activity and chemical composition of Algerian pomegranate (*Punica granatum* L.)

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**Key words:** *Punica granatum*, Peel extracts, Anti-inflammatory, Mice, Phenolic compounds

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## Abstract

*Punica granatum* L. is a plant widely used in traditional Algerian medicine to treat digestive, inflammatory and painful diseases. The objective of the present study was to determine the phenolic, flavonoids, anthocyanin, hydrolyzable & condensed tannins, proanthocyanidin contents and to evaluate in vivo the anti-inflammatory activity of the methanolic and aqueous extracts of the peel of *Punica granatum* fruit. Doses of 250 and 500 mg/kg of methanolic and aqueous extracts were administered orally in carrageenan-induced paw edema in mice, using Diclofenac (50 mg/kg) as a standard drug. Increases in paw diameter were measured for 6 hours at a 1-hour interval. After that, the mice were scarified and the inflamed paw tissue was removed and subjected to histopathological study. The results of the methanolic (ME) and aqueous (AE) extracts showed a significant inhibition (*** $p < 0.001$) of the mouse paw edema in a dose-dependent manner after 6 hours of carrageenan injection, compared to the control group. The percentages of edema inhibition of methanolic and aqueous extracts at a dose of 500 mg/kg were 80.72% and 51.94%, respectively, after six hours. These results were confirmed by the histological study, thus showing the presence of a less intense inflammatory infiltrate compared to the control group where the inflammation was more pronounced thus proving that carrageenin did induce an inflammatory reaction. This study revealed that peel extracts of *Punica granatum* have significant anti-inflammatory activity, which could be explained by the presence of a large amount of phenolic compounds.

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## Introduction

Inflammation is the response of an organism's immune system to the damage caused to its cells and vascularized tissues by microbial pathogens such as viruses and bacteria, as well as by injurious chemicals or physical insults (Mukhija and Sundriyal, 2013). Inflammatory responses are essential for the maintenance of normal tissue homeostasis (Ahmed, 2011). Although painful, inflammation is usually a healing response, but in some instances, inflammation proceeds to a chronic state, associated with debilitating diseases such as arthritis, multiple sclerosis, or even cancer (Weiss, 2002).

Inflammatory cells liberate a number of reactive species at the site of inflammation leading to exaggerated oxidative stress. On the other hand, a number of reactive oxygen/nitrogen species can initiate the intracellular signaling cascade that enhances proinflammatory gene expression (Anderson *et al.*, 1994; Flohé *et al.*, 1997). Thus, inflammation and oxidative stress are closely related pathophysiological events that are tightly linked with one another. In fact, experimental data show the simultaneous existence of low-grade chronic inflammation and oxidative stress in many chronic diseases like diabetic complications, cardiovascular and neurodegenerative diseases, alcoholic liver disease, and chronic kidney disease (Halliwell, 2006; Hald *et al.*, 2007; Onyango, 2008; Biswas, 2016).

In view of the considerable increase in the number of inflammatory pathologies and the side effects of synthetic anti-inflammatory drugs, many researchers from all over the world are joining in the search for compounds of vegetable origin which could alleviate these negative aspects.

Medicinal plants have long been considered useful in the development of new drugs and continue to play an important role in drug discovery processes. These plants are relatively inexpensive and available and their uses depend on ancestral experience. The majority of people in developing countries remain dependent on traditional plants for health care.

*Punica granatum* L., a species belonging to the Lythraceae family, is one of the most frequently used medicinal plants in Algeria due to its lack of knowledge of its therapeutic value. Pomegranate peels are characterized by an interior network of membranes comprising almost 26–30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins, and other complex flavonoids) and hydrolyzable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid). These compounds are concentrated in pomegranate peel and juice, which account for 92% of the antioxidant activity associated with the fruit (Negi *et al.*, 2003; Afaq *et al.*, 2005; Zahin *et al.*, 2010).

Several studies have demonstrated the anti-inflammatory, antimicrobial, antihelminthic, and antioxidant potential of the active ingredients of pomegranate extracts, suggesting their preventive and curative role in gastro-mucosal injuries and cancer chemoprevention (Al-zoreky and Nakahara,2003; Arun and Singh, 2012).

This study aims to evaluate the anti-inflammatory activity of methanolic and aqueous extracts of the fruit peel of *Punica granatum* L.

## Materials and methods

### *Plant material*

The fruit *pomegranate (Punica granatum* L.) *cultivar Doux de Messaad* was collected from the locality of Beni Tamou in the northern region of Algeria at harvest maturity in the beginning of October 2014, which is the normal ripening period for the pomegranate. It has been identified and authenticated at The National School of Agronomy (ENSA), El Harrach (Algiers). The species exist in the package number 35 at the herbarium of North Africa and a voucher specimen was deposited in the Department of Botany.

The fruits were harvested randomly from each of the four orientations of the trees while avoiding the fruits most exposed to the sun and also those that exist squarely down the tree.

After harvesting, the fruits are immediately and carefully stored in a refrigerator at a temperature of 4 °C until the time of analysis.

*Animals*

Swiss albino mice (25-30 g) were collected from the animal unit of Pasteur institute Algiers. The animals were housed in standard cages, in groups of six, at room temperature (25±3°C) and 12 h dark/light cycle, with both food and water *ad libitum*.

*Preparation of extracts*

*Methanolic extract (ME)*

The peels were manually removed, sun-dried and powdered. Approximately 400g powdered plant material was extracted with methanol using a Soxhlet extractor for 4 h. The extract was filtered through Whatman-1 filter paper for removal of peel particles and concentrated under vacuum at 40 °C. The marc was dried to dry under the high for 2 days; it will be used in the preparation of the aqueous extract.

Aqueous decoction of pomegranate peels (Hot- Water Extract). The marc (50g) was put into 500 ml of distilled water and boiled under reflux for 6 hours at a temperature of 200°C. After stirring, the cooled decoction was filtered through Whatman-1 filter paper, preceded by passage through a muslin cloth to avoid choking by vegetative debris. The separate marc was subjected to a second extraction using 50 ml of distilled water.

The collected supernatants were concentrated by a Tel Star lyophilizer at a temperature of -55°C and pressure of 0.10 bar.

*Phytochemical analysis*

A stock concentration of 1 % (W/ V) of each extract obtained using methanol and water was prepared using the respective solvent. Extracts were tested for the presence of active principles: tannins, alkaloids, phytosterols, triterpenoids, flavonoids, cardiac glycosides, anthraquinone glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats following standard methods (Harborne, 1998; Kokate, 2005; Roopalatha and Nair, 2013).

*Polyphenols analysis*

*Total polyphénols content (TPC)*

The Folin–Ciocalteu method was used for total polyphenol concentration determination, based on the optimized condition by Singleton and Rossi (1965) with slight modifications. The dry extracts of peel were diluted in a methanol-water mixture (6: 4 v/v). 300 µl of each extract is placed in a test tube was mixed with 1.5 ml of 10-fold-diluted Folin–Ciocalteu reagent and 1.2 ml of 7.5% sodium carbonate. The mixture was allowed to stand for 90 min at room temperature before the absorbance was measured by a PerkinElmer Lambda UV–Visible spectrophotometer at 760 nm. Gallic acid was used as a standard. The results were expressed as mg Gallic acid equivalent per gram of dry extract).

*Total flavonoids content (TFC)*

The total flavonoids content in each extract was determined spectrophotometrically according to the method of Quettier-Deleu *et al.* (2000) in Orak *et al.* (2012), using a method based on the formation of a complex flavonoid-aluminum, having the absorbtivity maximum at 430 nm. Quercetin was used to make the calibration curve. One milliliter of each sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a PerkinElmer Lambda UV–Visible and the flavonoids content was expressed as mg Quercetin equivalent per gram of dry extract.

*Total anthocyanin content (TAC)*

The TA was estimated by a pH differential method using two buffer systems: potassium chloride buffer pH 1.0 (25 mM) and sodium acetate buffer pH 4.5 (0.4 M) (Ozgen *et al.*, 2008). Briefly, 0.4 ml of each sample was mixed with 3.6 ml of corresponding buffers and read against water as a blank at 510 and 700 nm.

Absorbance (A) was calculated as: $A= (A_{510nm} - A_{700nm})\ pH_{1.0} – (A_{510nm} - A_{700nm})\ pH_{4.5}$

The TAC of samples (mg cyanidin-3-glucoside/g of extract) was calculated by the following equation: TA= [A x MW x DF x 100] x 1/MA

Where A: absorbance; MW: molecular weight (449.2 g/mol); DF: dilution factor (10); MA: molar absorptivity coefficient of cyanidin-3-glucoside (26.900).

*Total hydrolyzable tannins content (HTC)*

The content of hydrolyzable tannins was determined by the method of Willis and Allen (1998) with slight modifications. A mixture of one milliliter of each *Punica granatum* extracts (prepared in distilled water) and 5 ml of a 2.5 % aqueous solution of $KIO_3$ are mixed with a for ten seconds. Absorbance is obtained after incubation at ambient temperature and was measured at 550 nm using a PerkinElmer Lambda UV-Vis. Six concentrations of tannic acid (TA) ranging from 0.5 to 2 mg/ml were prepared under the same operating conditions as the samples. The results were expressed as mg of tannic acid equivalent per gram of dry weight.

*Total condensed tannins content (CTC)*

The content of condensed tannins in pomegranate extracts was determined to utilize the modified vanillin assay (Hagerman, 2002). Briefly, 1 ml of the sample (prepared in methanol) was added to 5 ml of the assay reagent (2.5 ml of the 1% vanillin solution mixed with 2.5 ml of the HCl solution 8% (8 ml HCl supplemented to 100 ml with methanol)), the mixture was vigorously stirred. 5 ml of the 4% HCl solution were added. The reaction mixture was kept in the dark for 20 min and its optical density was measured at 500 nm. Catechin was used as a standard, and the results were expressed as mg of catechin equivalents (mg CE/g).

*Total proanthocyanidin content (TPrC)*

Determination of Proanthocyanidins was based on the procedure reported in Li *et al.* (2006) and Wang *et al.* (2011). A quantity of 0.05 g of dried extracts was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin–methanol solution and 1.5 ml hydrochloric acid and the mixture were allowed to stand for 15 min at room temperature.

The absorbance at 500 nm was measured and the proanthocyanidins were expressed as catechin equivalents (mg CE/g of dry weight) using a catechin (0-0.08 mg/ml) standard curve.

*Acute toxicity test*

It was done according to Organization for Economic Co-operation and Development (OECD) guidelines 425 (2008). All extracts, namely methanolic and aqueous peel extracts in 250 and 500 mg/kg doses were administered to the mice orally (p.o.). All animals were observed for a period of 24 h after drug administration for behavioral changes, toxic reactions, and mortality.

*Anti-inflammatory activity*

In this part of the experiment, the anti-inflammatory activity of the pomegranate peel extracts was investigated on carrageenan-induced inflammatory paw edema (Winter *et al.,* 1962).

The methanolic and aqueous extracts of pomegranate peel was dissolved and dispersed in physiological saline (0.09%) and administrated by orally for a pretreated group of mice at 250 and 500mg/kg dosage. Physiological saline (0.09%) was given to the control group at the same volume as a vehicle. One hour after administration, 50µl of 1% carrageen solution was injected into the footpad of the hind paws of each mouse in all groups. Prior to carrageenan injection, the mice paw volume was measured with a Digital Caliper. Increasing of carrageenan-induced inflammatory paw volume was measured at 1, 2 3, 4, 5 and 6 h over the injection. The anti-inflammatory activity of *P. granatum* extracts was compared with that of 50 mg/kg Diclofenac. The percentage inhibition of the inflammation was calculated from the formula: inhibition %= $(D - D_t)/D_0*100$.

Where D is the diameter of injected paw, $D_0$ is the average inflammation (hind paw edema) of the control group of mice at a given time 0; and $D_t$ is the average of diameters of hind paw edema of the drug-treated (i.e. extract or reference Diclofenac) mice at the same time (Marzocco *et al.,* 2004).

*Histological procedure*

After the last measurement of the paw volume, all animals were anesthetized with diethyl ether and the paws were cut at lateral malleolus. Each sample was fixed in 10% formaldehyde solution. The sections were stained with hematoxylin and eosin (H&E) for the evaluation of the histological changes.

*Statistical Analysis*

Data obtained from this study were analyzed using Statistica software version 6.1 (StatSoft, Inc. USA, 2003). The results of phenolic contents are presented as the mean and standard deviation (SD). All the data of anti-inflammatory activity were expressed as mean ± S.E.M., and statistical analysis was carried out using one-way analysis of variance (ANOVA), followed by Dunnett's test.

**Results and discussion**

*Phytochemical analysis*

The phytochemical tests revealed that the various extracts of the peel (methanolic, aqueous) of *Punica granatum* are rich in secondary metabolites, in particular alkaloids, flavonoids, tannins, glycosides, carbohydrates, and saponins. The Ninhydrin, protein (Biuret), phytosterols and fats and fixed oils tests were negative in all extracts. The presence of these different phytoconstituents may be responsible for the therapeutic properties of the pomegranate.

**Table 1.** *Phytochemical screening of Punica granatum peel extracts.*

| Compounds | Test | Methanolic extract (ME) | Aqueous extract (AE) |
|---|---|---|---|
| Alkaloids | Dragendorff's | + + + | + |
| Flavonoids | Lead acetate | + + + | + + |
| Tannin | Gelatin | + + + | - |
| | Ferric chloride | + + + | + + + |
| Steroids et triterpénoids | Salkowski | + + + | + + + |
| Saponins | Foam | + + | + + + |
| Cardiac Glycoside | Keller-Killani | ++ | - |
| Hydroxyanthraquinone | | + | + |
| Carbohydrates | Fehling's | + | + |
| | Molish's | + + | + + |
| Phytosterols | Libermann-Burchard | - | - |
| Proteins | Biuret | - | - |
| Amino acid | Ninhydrin | - | - |
| Fixed Oils and Fats | | - | - |

+++: high presence; ++: moderate presence; +: low presence; - : absence.

The results of a phytochemical analysis are shown in Table 1. These results are in agreement with the results obtained by Hagir *et al.* (2016), which revealed the presence of triterpenoids, steroids, flavonoids, tannins, alkaloids, glycosides and saponins in the various extracts of fruit peel of *P. granatum*, namely the chloroform extract, methanolic extract and the aqueous extract. Similar results were also reported (Bhandary *et al.*, 2012; Hegde *et al.*, 2012; Uma *et al.*, 2012; Chebaibi and Filali, 2013; Moorthy *et al.*, 2013; Narasimha *et al.*, 2015 ; Sajjad *et al.*, 2015; Deore Leena *et al.*, 2016; Kesur *et al.*, 2016).

Also, Ozcal and Dinc (1994) indicate that flavonoids and tannins are the most abundant compounds in the peel of this plant. However, the chemical composition of the pomegranate and its products depends on the cultivar, crop area and climate, fruit maturity stage, cultural practices and production systems (Badenes *et al.*, 1998; Toor *et al.*, 2006; Raffo *et al.*, 2006; Borochov-Neori *et al.*, 2009; Zarei *et al.*, 2011).

*Polyphenols analysis*

All studies of phenol contents are shown in Table 2. The results showed that with methanol we obtained

the highest value of total phenolic compounds (227.92± 0.50 mg GAE/g; y = 0.0101x -0.028, $r^2$= 0.9911) whereas the lowest content was obtained in the aqueous extract (92.61±0.38 mg GAE/g). Our results are significantly higher than those found by Elfalleh *et al.* (2012) with 85.60 ± 4.87 and 53.65 ± 4.13 mg EAG/g for the methanolic and aqueous peel extracts, respectively. Moreover, these results are confirmed by those of Singh *et al.* (2002) who reported that the maximum antioxidant compounds yield was obtained with methanol compared to acetone and water.

**Table 2.** Mean values of total Phenolic compounds of pomegranate peel extracts.

| Samples | Total polyphenol (TPC) | Total flavonoids (TFC) | Total anthocyanins (TAC) | Hydrolysable tannin (HTC) | Condensed tannin (CTC) | Total proanthocyanidin ( TPrC) |
|---|---|---|---|---|---|---|
| | GAE mg/g dry weight | QE mg/g dry weight | CGE mg/g dry weight | TAE mg/g dry weight | CEmg/g dry weight | EC mg / g of dry weight |
| Methanolic extract | 227.92± 0.50 | 31.35±0.27 | 14.42±0.42 | 214.28±1.79 | 47.78±3.27 | 24.25± 0.24 |
| Aquoeus extract | 92.61±0.38 | 21.68±0.10 | 16.75±0.35 | 103.51±0.39 | 33.67±0.87 | 4.65± 0.07 |

Values are presented as means ± SD (n=3).

The total flavonoid content (Table 2) showed nearly similar values of 31.35±0.27 and 21.68±0.10 mg EQ/g (y = 0.038x + 0.018, $r^2$ = 0.999) for the methanolic and aqueous extracts of pomegranate peel, respectively. Shiban *et al.* (2012) showed that the composition of flavonoids in the methanol extract was 56.4 mg RE/ g. This is significantly higher than the value obtained (31.35 ± 0.27 mg QE / g). As reported by Souleman and Ibrahim (2016), aqueous extracts of peels from different Egyptian cultivars have varying flavonoid contents, ranging from 21.72 to 34.28 mg RE/g. These results are comparable to ours.

**Table 3.** Effect of methanolic and aqueous extracts of *P. granatum* fruit rind on anti-inflammatory activity (acute model).

| | | | Increase in paw thickness (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | Doses (mg/kg) | Initial paw thickness (mm) | 1h | 2h | 3h | 4h | 5h | 6h |
| Control | | 1.55±0.01 | 2.83±0.03 | 2.87±0.02 | 3.16±0.002 | 3.09±0.02 | 3.05±0.02 | 3.00± 0.03 |
| Diclofenac® | 50 | 1.55±0.01 | 2.79±0.04 | 2.61±0.01*** | 2.36±0.01*** | 2.26±0.01*** | 2.05±0.02*** | 1.82±0.02*** |
| Methanolic extract | 250 | 1.65±0.03 | 2.88±0.02 | 2.58±0.04*** | 2.26±0.01*** | 2.21±0.01*** | 2.07±0.01*** | 2.00±0.02*** |
| | 500 | 1.74±0.02 | 2.85±0.04 | 2.90±0.01 | 2.29±0.02*** | 2.19±0.01*** | 2.10±0.01*** | 2.06±0.02*** |
| Aqueous extract | 250 | 1.57±0.02 | 2.84±0.01 | 2.83±0.08 | 2.68±0.03*** | 2.59±0.02*** | 2.25±0.07*** | 2.09±0.06*** |
| | 500 | 1.47±0.01 | 2.65±0.02*** | 2.69±0.01** | 2.62±0.01*** | 2.27±0.01*** | 2.22±0.01 | 2.13±0.01*** |

Values are expressed in mean±SEM (n=6); **: P<0.001, ***: P<0.0001compared to the control (ANOVA, Dunnett).

Proanthocyanidins are oligomeric and polymeric end products of the flavonoid biosynthesis pathway. In addition to this diversity, polyphenols can be associated with various carbohydrates, organic acids and with each other. The existence of proanthocyanidins in common foods, including cereals, fruits, nuts, and spices, affects their texture, color, and taste. However, they are increasingly recognized as having beneficial effects on human health because of their powerful antioxidant capacity and their protective effects on human health; reduce the risk of chronic diseases such as cardiovascular disease and cancer (Dixon et *al.*, 2005; Prior et *al.*, 2005; Wissam *et al.*, 2012).

In this study, a quantitative estimate of the total proanthocyanidin content of the tested cultivar showed that the methanolic extract contains the highest value of proanthocyanidin then the aqueous extract (24.25±0.24, 4.65±0.07 respectively) mg CE/g, (the standard curve equation was y = 2.4552 x - 0.0012, $r^2$ = 0.9798).

These values are higher than those reported for five cultivars widely distributed in Egypt (0.085 to 0.339 mg CE/g for the methanolic extracts and 0.080 to 0.318 mg CE/g for the aqueous extracts) (Abdel-Hady, 2013). On the other hand, Middha *et al.* (2013) have also shown that the methanolic extract contains a more or less important proanthocyanidin content; 14.09 ± 1.56 whereas it is 9.09 ± 0.86 mg CE/g for the aqueous extract.

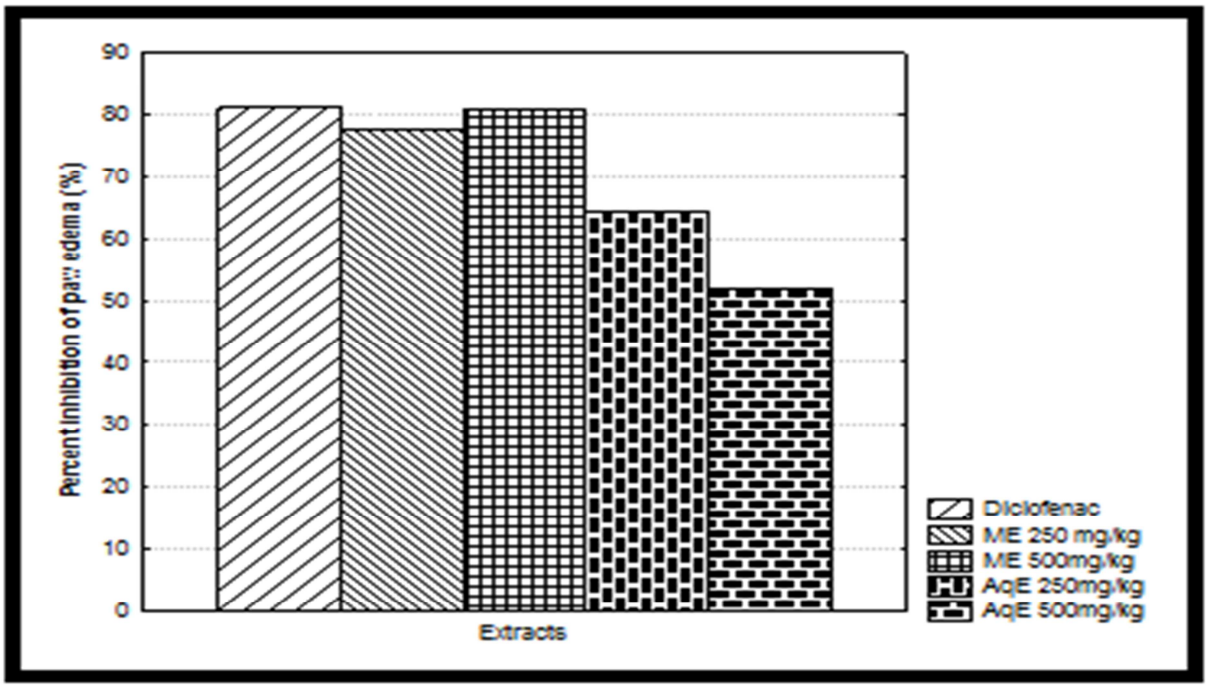


**Fig. 1.** Anti-inflammatory effect of methanolic and aqueous extracts of pomegranate peel (250 and 500mg/kg) in carrageenan-induced paw edema in mice. Percent inhibition of paw edema after 6h of treatment.

Furthermore, total anthocyanins content was 16.75±0.35 and 14.42±0.42 mg CGE/g for methanolic and aqueous extract, respectively. Indeed, anthocyanins are the primary source for the attractive red and red-purple colors of many fruits, including pomegranate arils and peels, and also exhibit considerable antioxidant activity (Seeram and Nair, 2002; Sehwartz *et al.*, 2009).

As for the tannins, total hydrolyzable tannins content was expressed as mg TAE/g of dry extract and varied according to extraction solvent uses methanolic extract (214.28±1.79) and aqueous extract (103.51±0.39) mg TAE/g (the standard curve equation was y = 0.2955 x+ 0.0784, $r^2$= 0.9933).

While, the methanolic extract is characterized by the highest content (47.78±3.27 mg CE/g) in condensed tannins (the standard curve equation is y =0.353 x - 0.0011, $r^2$= 0.9973).

These results clearly show that the hydrolyzable tannin fraction in the pomegranate fruit peel is greater than that of the condensed tannins. Saad *et al.*, (2013) also report this fluctuation between the levels of hydrolyzable tannins and condensed tannins in the pomegranate fruit peel of the various Tunisian cultivars. They explain this by genetic and environmental differences. On the other hand, they justify this fluctuation by distinguishing the thickness of the peel for each sample studied.

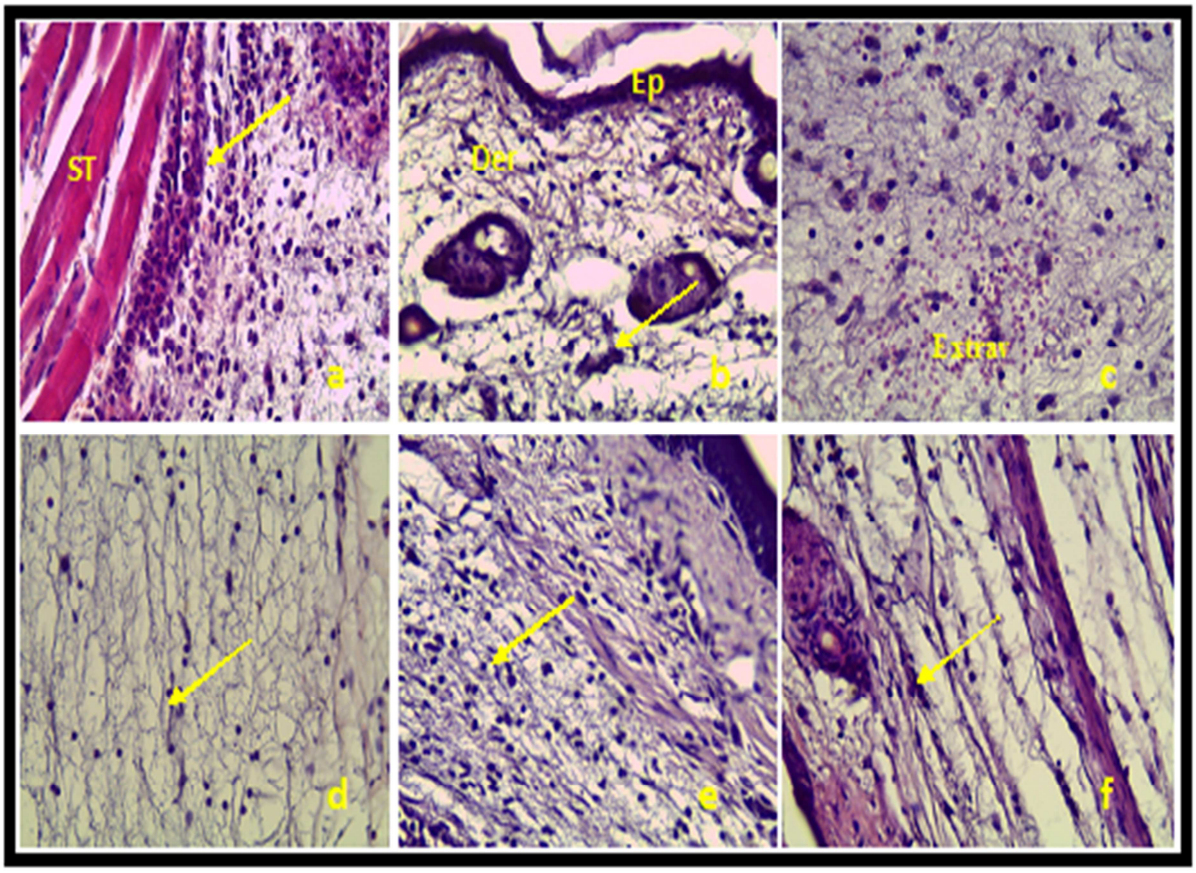


**Fig. 2.** Histopathologic examination of paw tissue of mice treated with; (a) Group I: normal mice, (b) group II: Diclofenac-treated mice, (c) group III: methanolic extract at 250mg/ml, (d) Groupe IV: Methanolic extract at 500mg/ml, (e) group V: aqueous extract at 250mg/ml, (f) Groupe IV: aqueous extract at 500mg/ml, 6h after intraplantar (i.p.l) injection of carrageenan (Carr). ST: striated muscle, Ep: epidermis, Der: dermis, Extrav: extravasation and →: inflammatory cell infiltration; G×40.

Finally, these results confirm the richness of *Punica granatum* fruit in phenolic compounds and which consists mainly of total polyphenols and hydrolyzable tannins.

*Acute toxicity*

After oral administration of the methanolic and aqueous extracts of pomegranate fruit peel 250, 500 mg/kg doses, no mortality was recorded for all animals observed 24h after drug administration.

*Anti-inflammatory activity*

An increase in mouse paw volume of all groups from the first hour of the experiment was observed. The carrageenan 1% induced edema which gradually increased to a maximum of 3.16 mm compared to the initial diameter mouse paw (Table 3).

The administration of Diclofenac at a dose of 50 mg/kg prevented significantly increasing the diameter of the mouse paw, which varies from 1.55 mm to 2.36 mm after 3 h of the carrageenan injection (Table 3).

The administration of the methanolic and aqueous extract at 250 and 500 mg/kg body weight significantly prevents acute paw edema in mousse after 6 hours (Fig.1.) (Table 3); however, the methanolic extract is more effective in preventing acute edema of the paws of the mice.

These results are consistent with previous work by Zhu *et al.* (2011) where the volume of edema following carrageenan injection increases with time until reaching its peak level by the third hour. The edematous development can be divided into two phases:

In the control group, from 0 to 3 hours, the edema caused by the phlogistic agent increases progressively and reaches a maximum at 3 hours (103.55%). From 3 to 6h: while the paw volume in the control group remains relatively unchanged, the changes in paw volume in the other experimental groups enter a period of decline, with a decrease demarcating a standard lot of others. Percentages of inhibition in edema increased from 52.61 to 17.82% for the group treated with diclofenac, respectively from the 3rd to the 6th hour.

In the first phase, from 0 to 3h, it has been reported that the vasodilation observed at the beginning of this phase, called "early" phase, is due to the release of histamine, serotonin and bradykinin mediators. According to Alam *et al.* (2011), edema is caused by secretion of 5-hydroxytryptamine (5-HT) during the first hour, followed by kinins to increase vascular permeability up to two and a half hours. As for the bradykinins which are produced by the cascade of plasma kinins at around 2:30, they would be responsible for the increase of the vascular permeability, thus causing an effusion of exudate into the inflammatory focus. These hemodynamic changes reached an increased level at the 3rd hour after carrageenan injection and then began to decline (Sharififar *et al.*, 2012). While the late phase occurs in 1 hour and lasts 3 hours or more, it is characterized by leukocyte infiltration and mediated only by prostaglandins (Vinegar *et al.*, 1969; Posadas *et al.*, 2004; Wang *et al.*, 2010).

Administration of Diclofenac sodium (50mg/kg) in the 2nd group showed a very significant decrease ($p<0.001$) in the evolution of inflammation in the legs of mice at the second hour, with a percentage of inhibition of 68.52% which was lower than that found in the negative control which was closed to 85%. Inhibition of the volume of edema reached a maximum threshold at the 6th hour after injection of carrageenan with a percentage of 80.97% (Fig.1.). NSAIDs act by inhibiting cyclooxygenase (COX), which inhibits the synthesis of prostaglandins, which gives this class of drugs its analgesic, antipyretic and anti-inflammatory properties (Pereira-Leite *et al.*, 2016).

NSAIDs may be classified as non-selective (inhibiting both COX-1 and COX-2) or selective COX-2 inhibitors (celecoxib, etoricoxib, meloxicam and parecoxib). However, most anti-inflammatory drugs are clinically effective in the second phase of inflammation (Olajid *et al.*, 2000; Mehmood *et al.*, 2016).

The evaluation of inhibition percent revealed that the methanolic extract in the two doses (250 and 500 mg/kg p.c.), as well as the aqueous extract at 250 mg/kg, produced anti-inflammatory effects from the second stage of development of edema, causing a very significant reduction in carrageenan-induced paws edema of mice. It is also noted that after 3 hours, the methanolic extract at 250 and 500 mg/kg showed a significant reduction in the volume of the paw of 64.37% and 69.72%, respectively, compared to the group treated with Diclofenac (49.19%). However, the aqueous extract at 500 mg/kg has anti-inflammatory activity from the first stage of development of the edema (early phase) compared to the negative control. The anti-oedematous activity in the late phase of the extracts could indicate that, like NSAIDs, they would have an inhibitory effect on the synthesis of prostaglandins, including the inhibition of COX. This could explain the mode of action of the active ingredients found in the peel extracts of the fruit of *Punica granatum*.

Our results are consistent with those of Labib and El-Ahmady (2015), which indicates that the anti-inflammatory effect of methanol extract (200 mg/kg b.w) similarly decreased in the group receiving the reference product Indomethacin (20 mg/kg b.w) after 1 h. After 2 hours, the ME group (200mg/kg) showed a significant decrease in edema volume of 53.3%, which was considerably higher than that resulting from the administration of Indomethacin (44, 86%).
On the other hand, these results are consistent with several studies showing that the anti-inflammatory activity of the extract can be explained in part by the presence in the fruit of polyphenolic compounds such as hydrolyzable tannins and flavonoids (Zarfeshany *et al.*, 2014).

The latter are well-known components of anti-inflammatory plants. Some flavonoids have shown inhibitory action in various animal models of inflammation. For example, some flavonoids have shown to inhibit animal models of acute inflammation: paw edema, ear edema and pleurisy (Kim *et al.,* 2017). The majority of the hydrolyzable tannins present in the grenades; gallotannins, ellagic acid, and gallagyl tannins, generally called punicalagins, has shown an inhibitory effect on the proliferation of human cancer cells and modulate inflammatory subcellular signaling pathways due to high antioxidant activity (Seeram *et al.,* 2005).

*Histopathology*

The histopathological investigation was conducted by hematoxylin and eosin staining. Biopsies of paws were taken from the following groups: negative control (saline, o.p), positive control (Diclofenac, 50mg/kg), the methanolic and aqueous extracts at 250 and 500mg/kg respectively 6h after i.p.l. carrageenan injection (Fig.2.).

The paw tissues of the control mice (group I) showed a polymorphic inflammation predominantly lymphoplasmacytic (Fig.2a.). Inflammation has spread to striated muscle tissue where there is a granuloma that contains polymorphic cells.

Tissue samples from the legs of Diclofenac-treated mice revealed a discrete inflammatory reaction (Fig.2b.); of less intensity than the control (group I). The evolution of the acute phase is quickly resolved by Diclofenac action.

The inflammatory infiltrate is observed in the paw tissues of the mice treated with the extracts at 250 mg/kg (Fig.2c & 2e.), but this infiltrate is less intense than the control group. Whereas, it's more intensive than diclofenac group. However, treatment of mice at 500mg/kg exhibited a significant decrease in the number of cellulars infiltrates (Fig.2d & 2f.).

In the present study, we showed that the pomegranate peel extracts produced anti-inflammatory effects in carrageenan-induced mousse paw edema dose-dependently.

Our results confirmed previous findings that pomegranate peel extracts exhibit a noticeable anti-inflammatory effect in experimental models (Mo *et al.,* 2013; Mansouri *et al.* 2015).

**Conclusion**

*Punica granatum* showed anti-inflammatory power, similar to those observed for non-steroidal anti-inflammatory drugs, such as Diclofenac, inhibiting edema and reducing the cellular infiltrates. It is also suggested that the mechanism of action of *Punica granatum* might be associated with the inhibition of inflammatory mediators. However, further studies are needed to isolate and characterize anti-inflammatory bioactive compounds present in both methanolic and aqueous extracts of *Punica granatum* fruit peel.

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