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Research Article

IN VIVO ANTI-INFLAMMATORY ACTIVITY AND POLYPHENOLIC CONTENT OF AQUEOUS AND ETHANOLIC EXTRACTS OF *FICUS CARICA* L. FRUIT

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ABSTRACT. The present study aims at studying the phytochemical compounds and evaluating *in vivo* the anti-inflammatory effect of aqueous and ethanolic extracts of *Ficus carica* L. fruit. The total contents of phenolics, flavonoids, and condensed tannins were determined spectrophotometrically using Folin-Ciocalteu, aluminum trichloride and vanillin reagents respectively. The *in vivo* anti-inflammatory activity was realized by paw edema model in mice induced by intraplantar injection of λ-carrageenan and treated with doses of each extract at 250, 350, and 500 mg/kg body weight. Paws' tissues were subjected to histological study to devote the effect of extracts at the tissue scale. Preliminary phytochemical screening indicates a significant appearance of flavonoids, tannins, alkaloids and steroids in both types of extracts. The aqueous extract marks the highest values in total phenolics, total flavonoids and condensed tannins contents: 951.06±61.08 mg GAE/100g dry weight (DW), 428.34±15.42 mg QE/100g DW and 474.07±50.25 mg CE/100g DW respectively. Our results show that both treatments with aqueous and ethanolic extract present a significant inhibition $^{*}P<0.05$ of the edema in a dose-dependent manner compared to the standard group (treated with 2-[2-(2,6-dichloroanilino) phenyl]acetic acid as anti-inflammatory medicine) during the whole experiment period. This was confirmed histologically by the observation of a less intense inflammatory infiltrate as the dose of extracts increase. This study reveals a pronounced anti-inflammatory effect in *Ficus carica* L. fruit extracts which could be related to the high amounts of phenolic compounds. Thus, fruit can be a promising natural substitute therapeutic to treat inflammation.

Keywords: *Bioactive compounds, mice, histological study, inflammation.*

INTRODUCTION

Inflammation is the set of reactionary defense mechanisms by which the body crushes and eliminates all substances that are odd to it like microbial attack and infection agents [1].

Inflammatory process is an organism's natural response characterized by activation of immune cells and increase liberation of pro-inflammatory mediators like cytokines, Tumor necrosis factor-α (TNF-α), interleukin (IL)-1β and interleukin IL-6 intensifying the inflammatory response and acting as speeder up of the response process to reduce inflammation [2].

The inflammatory reaction sometimes surpasses its destinations in case of chronic inflammation and causes harmful impacts such as tissue damage, cardiovascular diseases and chronic arthritis or even cancer [1, 3].

Anti-inflammatory medicines, both steroidal and non-steroidal (SAIDs and NSAIDs), are frequently used to treat inflammation, but they can become harmful and damaging to the organism due to its side effects and cause disorders such as gastric lesions and other digestive system upset [4]. This leads researchers to find natural alternatives with bio-pharmacological interest issue especially from medicinal plants due to the scarcity of their harmful effects on health.

Crude extracts of plants represent a source of natural bioactive molecules such as polyphenols known by their therapeutic proprieties. They are being studied for their potential application as an alternate treatment of infectious and inflammatory disorders [5].

Algeria, rich by its biodiversity, its climate and its flora with 3183 species [6] is a very important geographical platform and deserves to be explored in the field of the research of anti-inflammatory molecules stemming from plants which have for a long time been used by a large part of the population as an inescapable means of food and medicine.

Ficus carica L. is a nuraceutic fruit from the Moraceae family [7] known for its therapeutic properties such as gastrointestinal, respiratory, cardiovascular and antipyretic which have been shown to be effective in treatment of inflammation, paralysis and other disorders [8, 9].

Figs were analyzed for its phytochemical compounds and presence of total polyphenols, flavonoids, tannins, saponins, alkaloids, coumarins, terpenes, anthocyanins were detected in its extracts [10, 11].

Palmeira et al. [12] confirmed presence of macronutrients, free sugars, tocopherols, organic and fatty acids in significant quantities in Portuguese fig variety.

Despite the fact that there is a several body of literature on the composition of *Ficus carica* L. fruits, only a few studies shed light on the study of its anti-inflammatory effect *in vivo* considering that the bioactive compounds content differs depending on the environmental conditions, cultivar and genotype [13].

Thus, to expand the knowledge of fig phenolic compounds and to valorize it as a nuraceutic fruit, the main purpose of this study is to evaluate the biochemical content and the anti-inflammatory activity of aqueous and ethanolic extracts from Algerian dark variety of *Ficus carica* L. fruit in mice model at doses of 250, 350, and 500 mg/kg body weight (b.wt), and to compare them and find the best extract in terms of richness in bioactive compounds and efficiency against inflammatory disorders.

MATERIALS AND METHODS

Standards and reagents

Ethanol, hydrochloride acid, methanol, chloroform, diethyl ether, acetone and toluene were from Honeywell Riedel-de haën (Germany); magnesium metal, bismuth nitrate, potassium iodide, Gallic acid (GA), Folin-Ciocalteu (FCR), Quercetin, vanillin, Catechin, sulfuric acid, sodium hydroxide, λ-carrageenan, eosin and hematoxylin were from Sigma Aldrich (USA); Sodium carbonate, aluminum chloride and ferric chloride were from VWR chemicals (USA). 2-[2-(2,6-dichloroanilino) phenyl]acetic acid was from Biocare laboratory (Algeria). Sodium chloride 0.9% was from Biolyse laboratory (Algeria).

Plant material

Ficus carica L. fruits were harvested from Aghbal, Tipaza-Algeria at their full mature stage in August 2020. The variety had dark skin color and locally known as Azendjar (AZ). Botanical identification was confirmed by experts of ITAFV (Technical Institute for Fruit Trees) Algiers. A voucher specimen (UM/09/2020) was placed at the herbarium, Department of Biological Sciences, Mostaganem University-Algeria.

Drying procedure

The fruits were exposed to sun light for 10 days then transferred to laboratory, chopped with an electric chopper and lyophilized (CHAIST LOC-2M, Germany) at -55 °C under vacuum (0.1 bar) for 48 hours. The fruits were crushed using an electric grinder. The obtained powder was conserved in shaded hermetically sealed glass boxes at -20 °C until usage [14, 15].

Aqueous extract (AQE) preparation

The powder was extracted by soaking with distilled water (1/5; w/v) (weight/volume) for 24 h at 25 °C with magnetic agitation 300 rpm. The extract was filtered with a muslin cloth (0.25 mm pores size) then lyophilized (CHAIST LOC-2M, Germany) at -55 °C under vacuum (0.1 bar) and stored at -86°C until uses (Extraction yield = 11.46%) [16].

Ethanolic extract (EE) preparation

The powder was soaked in ethanol 80% (1/4; w/v) with stirring 300 rpm at room temperature for 72 h. A muslin cloth (0.25 mm) and Whatman filter paper N°1 were used to filtrate the homogenate. Extraction procedure was duplicated and the filtrates were combined and concentrated with a rotary evaporator under vacuum at 45 °C to obtain a thick paste of dark brown color which was gathered and saved at -20 °C until uses (Extraction yield = 9.95%) [11].

Preliminary phytochemical screening

The extracts were submitted to a test series for identifying the main groups of phytochemical compounds [17, 18].

Test for flavonoids (Shinoda's test): to 0.2 g of the extract, 2 ml of methanol were added, the mixture was slightly warmed then filtered and a chip of magnesium metal was added to the filtrate with 3 drops of concentrated HCl (12 M). Flavonoids are present when the color is pink, red or orange.

Test for alkaloids (Dragendorff's test): in stream bath, 1 g of cruds extracts was dissolved in 10 ml of 1% (v/v) HCl water solution. After filtration, few drops of the Dragendorff reagent which consists of potassium iodide-bismuth nitrate were added to the mixture. Appearance of turbidity or precipitations reveals the existence of alkaloids.

Test for steroids determination (Salkowski's reaction): in 6 ml of chloroform, 1g of the extracts was dissolved and few drops of concentrated sulfuric acid were added. Steroids are present as a red-brown layer at the interface appears.

Test for tannins: 5 ml of distilled water were added to 0.25 g of plant. After filtration, few drops of ferric chloride solution at 1% were added. Occurrence of blue-black or blue-green precipitate reveals presence of tannins.

Quantitative analyses : Total phenolic content (TPC)

Folin-Ciocalteu reagent was used to quantifying The TPC [19]. 200 µL of the extract was mixed with 800 µL of FCR (1/10 dilution in distilled water). After 5 min, 1000 µL of carbonate of sodium at 7.5% (w/v) was included. Absorbance was detected at 765 nm after 1 hour of incubation at (25 °C). For the calibration curve, Gallic Acid was utilized as the standard, and the findings were expressed in mg of GA equivalents (GAE)/100 g dry weight DW. All quantification was repeated in triplicate (R^2= 0.9991).

Total flavonoid content (TFC)

Aluminum chloride was utilized for quantifying TFC [20]. One ml of every extract or standard was combined with the same volume of 2% aluminum chloride solution (w/v) prepared in methanol. The reaction mixture was incubated in darkness at (25 °C) for 30 min and the absorbance reading was done at 430 nm against a blank. For calibration, Quercetin was employed as standard and the TFC were expressed in mg of Quercetin equivalents (QE)/100 g DW (R^2= 0.991).

Condensed tannin content (CTC)

The measurement of CTC was performed with Vanillin-HCl technique [21]. 0.4 ml of extract received 3 ml vanillin of 4% (w/v) produced in methanol. The mixture was shaken and immediately 1.5 ml of HCl was added. The mixture is stirred again and kept on darkness for 20 min and the absorbance was taken at 550 nm against blank. Catechin was used as standard for calibration curve and the CTC were expressed as mg of Catechin equivalents (CE)/ 100 g DW. (R^2 = 0.998).

Animal experiments

Forty female NMRI mice (25-31 g) were purchased from Pasteur Institute of Algeria (PIA) and were transported to animalery of Faculty of Sciences (Mostaganem University, Algeria). The mice were kept in cages made of polypropylene (55×33×19 cm) and submitted for adaptation under a controlled and specific-pathogen free environment (CSPFE) for 14 days. Standard housing conditions: (temperature of 23 ± 4 °C, 12:12 light: dark light cycle system), ad libitum access to water and food that was provided as dry pellets.

The assay was conducted in accordance with the European Communities Council Directive (2010/63/EU) for animal experiments and the protocol employed was approved by the biology department (University of Mostaganem) under inscription number 01/SNV/21.

Acute toxicity test ATT

The test of acute toxicity was realized as described by Organization for Economic Co-operation and Development (OECD) guidelines 425 [22]. This test entails gradually increasing extracts doses administrated to the mice by intra gastric gavage (i.g.g.). Forty mice were divided into eight groups; each group contains five animals of similar mean of body weight as below.

Groups I, II: mice received a dose of 250 mg/kg (b.wt) of AQE and EE respectively by i.g.g.

Groups III, IV: mice received a dose of 350 mg/kg (b.wt) of AQE and EE respectively by i.g.g.

Groups V, VI: mice received a dose of 500 mg/kg (b.wt) of AQE and EE respectively by i.g.g.

Groups VII, VIII: mice received a dose of 2000 mg/kg (b.wt) of AQE and EE respectively by i.g.g.

All mice were observed for any eventual signs of toxicity as convulsion, limb paralysis, and increase in locomotor activity, salivation, diarrhea, coma or mortality. Observations were noticed after 30 min of i.g.g. of extracts, every 1 hour for the first 6 hours and every day for 14 days [23].

In vivo anti-inflammatory activity

The anti-inflammatory activity of *Ficus carica* L. fruit extracts was examined by the model of injection of λ-carrageenan in the paw [24]. Forty mice were fasted for 16 h before experiment. Mice were divided into eight groups and received the vehicle or plant extracts by i.g.g. as below:

Group I: positive control, mice received NaCl solution (0.9%) i.g.g. b.wt.

Group II: standard STD group, mice received 2-[2-(2,6-dichloroanilino) phenyl]acetic acid at 50 mg/kg dissolved in 0.9% NaCl i.g.g.

Groups III, IV: mice received a dose of 250 mg/kg (D1) of b.wt of AQE and EE respectively dissolved in 0.9% NaCl by i.g.g.

Groups V, VI: mice received a dose of 350 mg/kg (D2) of b.wt of AQE and EE respectively dissolved in 0.9% NaCl by i.g.g.

Groups VII, VIII: mice received a dose of 500 mg/kg (D3) of b.wt of AQE and EE respectively dissolved in 0.9% NaCl by i.g.g.

After one hour of i.g.g., edema was induced by intraplantar injecting of 0.1 ml of 1% λ-carrageenan solution (freshly prepared in NaCl 0.9%) into the right hind paw. The paw volumes were measured using caliper 1h before the λ-carrageenan injection and every hour for 6 hours.

The percentage of inhibition of edema (INH%) was calculated passing by the calculation of the percentage of augmentation of edema AUG% according to the following formula [25]:

$$\%\, \boldsymbol{AUG} = \frac{\boldsymbol{V_t - V_0}}{\boldsymbol{V_0}} \times \mathbf{100}$$

$\boldsymbol{V_t}$: the volume of paw at T time; $\boldsymbol{V_0}$: the volume of paw at T0 before injection of λ-carrageenan.

$$\%\boldsymbol{INH} = \frac{\%\ \mathbf{AUG\ control} - \%\ \mathbf{AUG\ treated}}{\%\ \mathbf{AUG\ control}} \times \mathbf{100}$$

Histological examination

After all paw volume measurements, mice were euthanized by diethyl ether and paws were cut at lateral malleolus and fixed in formaldehyde solution 10% then were subjected to treatment with HCl decalcifying solution to remove cartilage and bones.

To perform the histological study, paraffin was used to embed the samples and the Hematoxylin–eosin (H&E) staining was used to color the tissue sections of 4μm

thickness. The microscopic assessment was realized with microscope (Leica, Germany) and digital slide photography.

Statistical analyses

The statistical data were presented as mean ± standard deviation (SD) and as mean ± standard error of mean (SEM) for the phytochemical analyses results and the *in vivo* study respectively. Analysis of variance was used to conduct the research (ANOVA) and significance was developed with least significant difference (LSD) post hoc treatment with XLSTAT by Addinsoft (2020) software. Values of $P < 0.05$, $P < 0.01$, $P < 0.001$ were considered significant (*), highly significant (**) and very highly significant (***), respectively.

RESULTS AND DISCUSSION

Phytochemical screening

The results of qualitative phytochemical screening of *Ficus carica* L. fruit extracts are presented in Table 1. The finding revealed that the various extracts of the fruit (aqueous, ethanolic) are rich in secondary metabolites, in particular flavonoids and tannins with very highly presence in the AQE. The results are in agreement with those of Alqethami and Aldhebiani [26] on presence of alkaloids and tannins except for flavonoids that were absent in their sample.

Table 1. *Preliminary phytochemical screening of Ficus carica L. fruit extracts.*

Compounds	Test	Aqueous extract	Ethanolic extract
Flavonoids	Shinoda	+++	++
Tannins	Ferric chloride	+++	++
Alkaloids	Dragendorff	+	+
Steroids	Salkowski	+	+

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

The polyphenolics are the major secondary metabolites found in plants, their content varies depending on many factors such as cultivar, crop, climate, soil and maturity stage [27].

Table 2 demonstrates results of total phenolic, (TPC), total flavonoid (TFC) and condensed tannin content (CTC). In this study the AQE was marked the highest values in TPC, TFC and CTC with the average of: 951.06 ± 61.08 GAE mg/100 g DW, 428.34 ± 15.42 QE mg/100 g DW, and 474.07 ± 50.25 CE mg/100 g DW respectively compared to the EE with $^{***}P<0.001$ for TPC and TFC and $^{**}P<0.01$ for CTC.

Table 2. *Amount of total phenolic, total flavonoids and condensed tannins content in aqueous and ethanolic extracts of Ficus carica L. fruit*

Samples	Total phenolic compounds (TPC)	Total flavonoids compounds (TFC)	Condensed tannin compounds (CTC)
	GAE mg/ 100 g dry weight DW	QE mg/ 100 g dry weight DW	CE mg/100 g dry weight DW
Aqueous extract	$951.06 \pm 61.08^{***}$	$428.34 \pm 15.42^{***}$	$474.07 \pm 50.25^{*}$
Ethanolic extract	403.66 ± 32.11	24.64 ± 0.19	277.77 ± 29.39

The data are expressed as mean ± SD. $^{*}P<0.05$ indicates significant outcomes, $^{**}P<0.01$ indicates highly significant outcomes and $^{***}P<0.001$ indicate very highly significant outcomes against ethanolic extract.

The richness of the fruit extracts with TPC is confirmed by the results obtained by Yang et al. [28] who found the content of 12.5 mg/g for AQE. Works elaborated by Bachir Bey and Louaileche [29] on nine varieties prepared with hydro-acetone extract gave values from 482.62 to 644.11 mg/100 g DW. Results marked values varies between 1120 to 2681.8 mg GAE/100 of DW in methanol/acetic acid extract from some dried fig fruit cultivars grown in Iran [30].

Our results are superior to those found by Rtibi et al. [16] who found the content of 436.64±7.65 GAE/100 g in AQE obtained by the same extraction procedure. The high yield of the AQE compared to other extracts prepared with different extraction solvents was confirmed by the work elaborated by Debib et al. [31] with an amount of 756.65±16.65 mg GAE/100 g, followed by methanolic extract with 426.12±10 mg GAE/100 g and acetone extract with 256.28 mg GAE/100 g whereas, Mopuri et al. [32] found the average of 104.67±5.51 mg GAE/g for EE and 66.6±1.63 mg GAE/g for AQE.

About the TFC; our findings are superior to those reporting the highest value in the methanolic extract with 137.4 mg CE/100 g [33]. Value of TFC in EE was 5.15 mg CAE /g [34]. Rtibi et al. [16] found the value of 50.61 ± 4.12 mg EC/100 g in the AQE.

For CTC, our results of EE are in line with those found by Benmaghnia et al. [35] reporting a value of 254.1±0.43 mg CE/100 g DW in AQE whereas the EE was marked the value of 7.05±0.3 mg CE/100 g DW.

In vivo anti-inflammatory activity

Medicinal plants are the major alternative candidate to treat various diseases with limited side effects. Many researchers report the several biological activities of *Ficus carica* L. fruit [36]. Our study put the light on the evaluation *in vivo* of the anti-inflammatory effect of its fruit extracts with mouse paw edema model.

The intra gastric gavage of AQE and EE at the doses of 250, 350, 500 and 2000 mg/kg (b.wt) didn't cause any signs of acute toxicity (AT) or even mortality during the 24 h of observation as well as during the 14 days follow up period.

2-[2-(2,6-dichloroanilino) phenyl]acetic acid: an NSAIDs drug was used as reference (standard STD) for comparing its effect in relation to different doses of *Ficus carica* L. fruit extracts on the changes marked on the legs of the mice.

The percentage of the inhibition of edema (INH%) in mice treated with the AQE is presented in (Fig. 1A).

At 1-hour post λ-carrageenan injection, INH% was lower in the group treated with 2-[2-(2,6-dichloroanilino) phenyl]acetic acid (14.56±8.51) %. This inhibition was accentuated with time to reach (83.68±11.23) % at the 6th hour of the experiment.

INH% of paw edema was important in groups treated by AQE. It marked (33, 12±10, 17) % at the 1st hour after the injection of carrageenan and reached (91, 22±10, 69) % at the 6th hour in group treated by 250 mg/kg b.wt dose. Group that received 350 mg/kg b.wt marked a highly significant INH% ($^{**}P<0.01$) the first four hours compared to the standard. It reached (98.18±7.16) % with ($^{**}P<0.05$) at the 6th hour. Mice treated by 500 mg/kg b.wt marked the highest INH% of edema with ($^{***}P<0.001$) from the 1st to the 5th hour, it reached (99.34±1.94) % at the 6th hour with ($^{*}P<0.05$) compared to standard.

The treatment of inflammatory edema by EE marked its significant power compared to the standard from its 2nd dose (350 mg/kg b.wt) with ($^{*}P<0.05$) at the 1st, the 3rd and the 4th hour; ($^{**}P<0.01$) at the 5th hour and ($^{***}P<0.001$) at the 2nd hour. Dose of 500 mg/kg b.wt presented also a very important inhibitor power compared to the standard with ($^{***}P<0.001$) during the experiment except at the 3rd and the 6th hour where INH% marked a highly significant inhibition with ($^{**}P<0.01$) and ($^{*}P<0.05$), respectively against the standard as illustrated in (Fig. 1B).

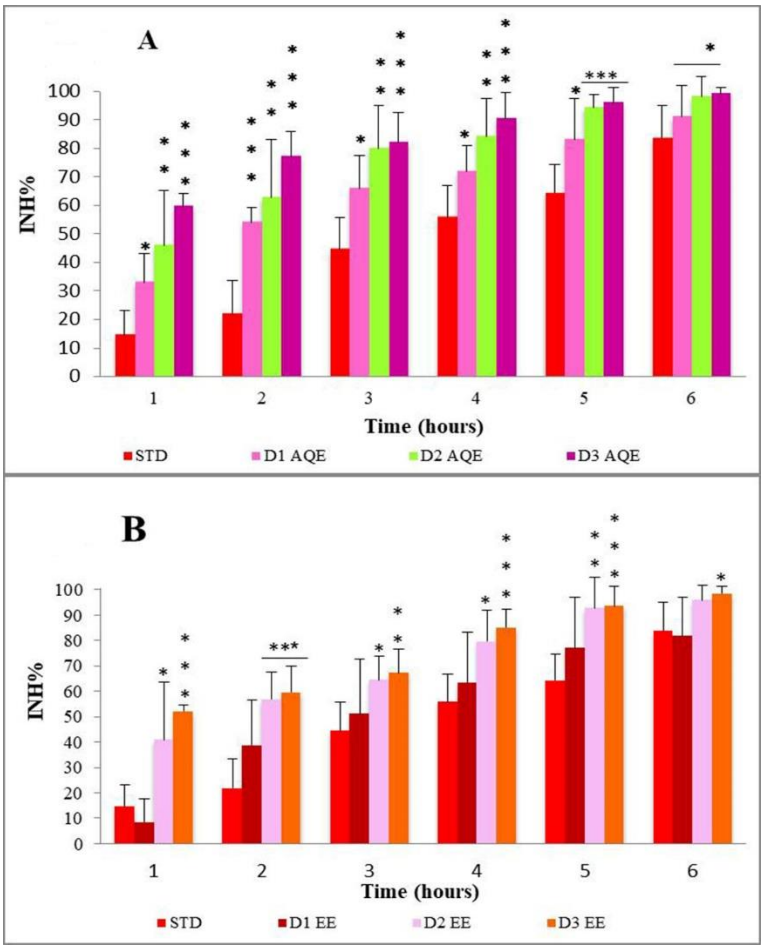

Fig. 1. *Percentage of inhibition of paw edema in mice treated by (a) aqueous, (b) ethanolic extract of Ficus carica L. fruit after 1,2,3,4,5 and 6 h of λ-carrageenan injection with D1: 250mg/kg b.wt; D2: 350 mg/kg b.wt; D3: 500 mg/kg b.wt. $^{*}P<0.05$, $^{**}P< 0.01$, $^{***}P<0.001$ against the standard (STD) group.*

Fig. 2 displays a comparative histogram of INH% of paw edema in mice treated by AQE and EE.

Both extracts shows almost a similar INH% except at the 1st hour when dose of (250 mg/kg b.wt) of AQE marked a highly significant INH% ($^{**}P<0.01$) of edema. Dose of (500 mg/kg b.wt) marked highly significant inhibition ($^{**}P<0.01$) at the 1st hour and a significant inhibition ($^{*}P<0.05$) at the 2nd and the 3rd hour compared to the same doses of EE.

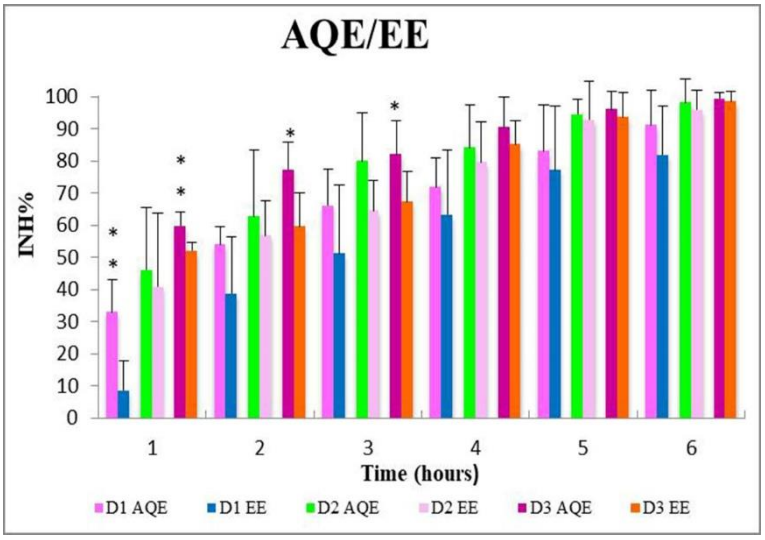

Fig. 2. *Comparison percentage of inhibition of edema between mice treated with aqueous and ethanolic extract of Ficus carica L. fruit after 1,2,3,4,5 and 6 h of λ-carrageenan injection with D1: 250mg/kg b.wt; D2: 350 mg/kg b.wt; D3: 500 mg/kg b.wt* $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

The measurement of edema is an excellent tool for quantification of subcutaneous inflammation induces by intraplantar injection of λ-carrageenan in mice that is standard, practical and widely used model for evaluation of anti-inflammatory properties of different factors [37, 38, 39].

The intraplantar injection of λ-carrageenan in mice triggers a mechanism of action marked by the liberation of chemical mediators involved in the inflammatory response which is biphasic. Phase 1: lasts for two hours immediately after induction of inflammation marked by a vasodilatation following a release of histamine and serotonin [40, 41]. This explains the low INH% in all groups at the 1st post λ carrageenan injection hour compared to the other times of the experiment. Phase 2: lasts up to five hours after phase 1 characterized by the synthesis of leukotriene, bradykinin and prostaglandin (PG) which is a major inflammatory mediator synthesized by the activation of cyclooxygenase pathway.

However in our study, the time progression observed in INH% during this phase in all mice treated can be translated by the inhibition of synthesis of these mediators [40, 41, 42].

The light augmentation in INH% at the 1st hour followed by its significant progression at the end of the experiment in mice treated with 2-[2-(2,6-dichloroanilino) phenyl]acetic acid is reflected by its late effect which is triggered 2 h after its i.g.g. That correspond its mechanism of action, which passes by inhibition of PG by inhibiting the two subtypes of

cyclooxygenase COX-1, COX-2. Our results are in line with those of Mansour et al. [43]. who worked with the same dose of the standard.

The edema was significantly reduced after treatment with fruit extracts compared to the standard in most times of the experiment.

Our results are superior to those of Singh et al. [44] reporting the significant dose-dependent anti-inflammatory effect of EE in cotton wool granuloma model with INH% of 25.83% and 40.85% at 250 and 750 mg/kg b.wt respectively. Results of Patil and Patil. [9] report the best anti-inflammatory power of leaves EE at 600 mg/kg b.wt with INH% of 75.90% and 71.66% for acute and chronic inflammation in rats respectively.

Also Ali et al. [45] report the significant anti-inflammatory effect of leaves EE in rats following the same model of inflammation induction where INH% reached 48.88%, 56.66% at 100 and 200 mg/kg respectively.

The reduction of edema in the mice treated by our extracts can be explained by the inhibition of inflammation mediators due to the presence of bioactive compounds revealed during their dosage sharing the same 2-[2-(2,6-dichloroanilino) phenyl]acetic acid anti-inflammatory pathways.

The presence of polyphenols in different parts of *Ficus carica* L. plant as fruits, latex, leaves; is responsible of analgesic and anti-inflammatory activities [46].

Many flavonoids and polyphenols have been reported for their antioxidant, anticancer, cardio-protective, anti-inflammatory proprieties [47]. Likewise; phenolic, tannins and alkaloid content was studies for their therapeutic potential with respect to inflammatory troubles [48].

The Anti-inflammatory power of TPC is suggested to be linked to the regulation of cellular activity in inflammatory cells and to the modulation of the activities of phospholipase A2, COX, lipoxygenase (LOX) involved in arachidonic acid metabolism as well as the modulation of arginine metabolism enzymes, suppressing clooxygenase-2/nitric oxide synthase and functioning as free radical scavengers to limit the release of additional pro-inflammatory mediators [49, 41].

Histological study

Biopsies of paws were taken from all groups of experiment compared to healthy paw tissues used as negative control to distinguish the normal from the pathological state Fig.3.

Tissues of positive-control group present a polymorphous inflammatory infiltrate, bullous lesions with lymphocytic dispersion in the tissue surface.

2-[2-(2,6-dichloroanilino) phenyl]acetic acid group presented a partial disappearance of the edema and a decrease in the intensity of the inflammatory infiltrate.

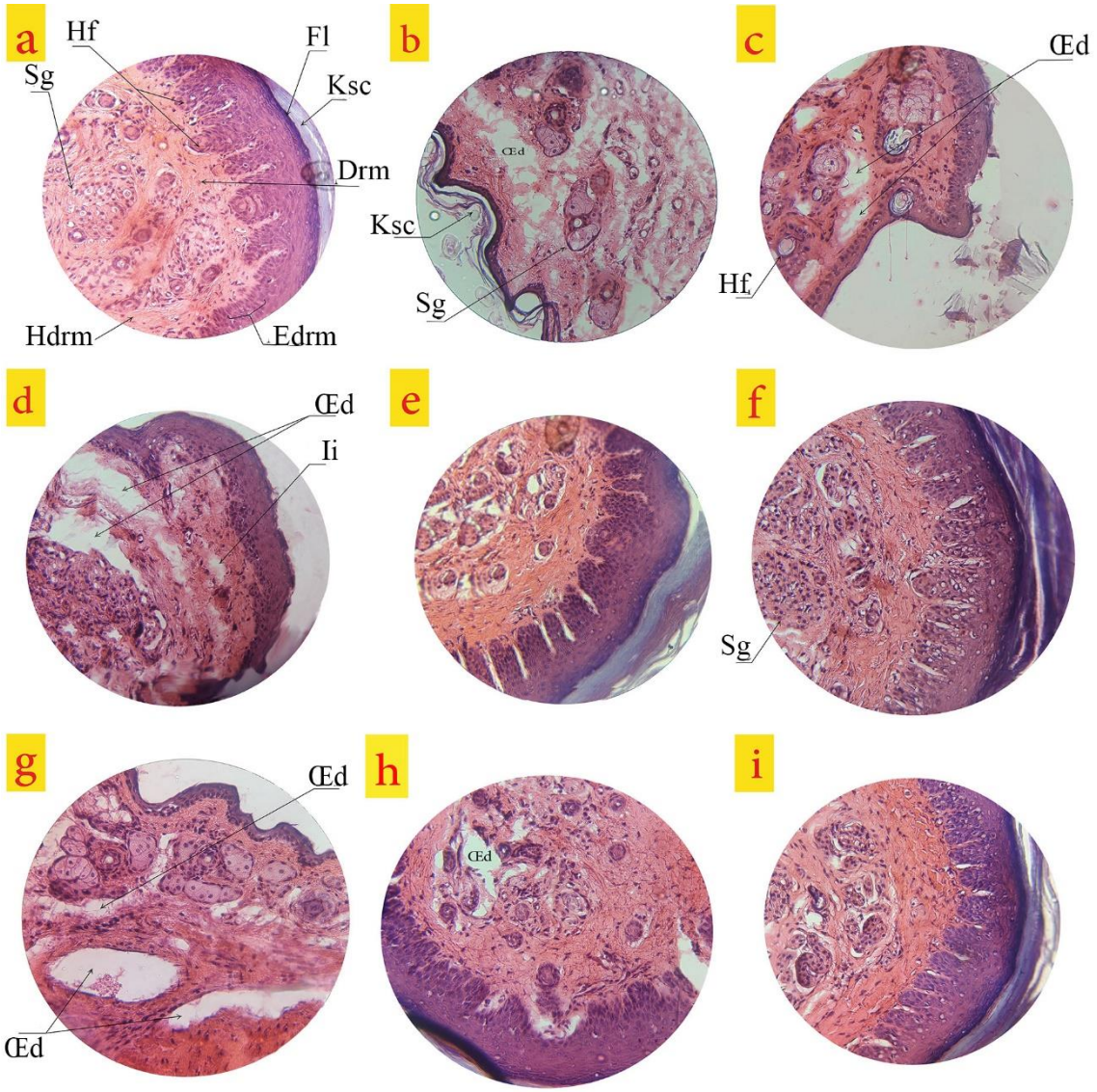

Fig. 3. *Representative micrographs showing the effect of different doses of Ficus carica L. fruit extracts on the λ-carrageenan induced edema on mice paws compared to: negative control, positive control, and the standard (X40) with H&E coloration.* ***a, b, c:*** *negative, positive and 2-[2-(2,6-dichloroanilino)phenyl]acetic acid control,* ***d, e, f****: D1(250 mg/kg) b.wt, D2(350 mg/kg) b.wt, D3 (500 mg/kg) b.wt of AQE,* ***g, h, i****: D1(250 mg/kg) b.wt, D2(350 mg/kg) b.wt, D3 (500 mg/kg) b.wt of EE respectively.* ***Hf****: hair follicle****, Ksc****: keratinized stratum corneum,* ***Sg****: sebaceous gland,* ***Œd****: edema, Ii: inflammatory infiltrate,* ***Hdrm****: hypodermis,* ***Edrm****: epidermis,* ***Drm****: Dermis,* ***Bl****: basal layer,* ***Fl****: flattened layer.*

The tissues of mice treated with the extracts at 250 mg/kg b.wt presented a slight edema with presence of congestion in the dermis. While treatment by the extracts at 350 mg/kg b.wt generates a recovery observed at the dermis with few lymphocytes less edema compared to 250 mg/kg b.wt dose.

Absence of edema with presence of some rare lymphocytes migrating towards the epidermis with a normal appearance of the different layers of the skin was observed in tissues of mice treated with 500 mg/kg b.wt of both extracts.

The research backs up previous claims that *Ficus carica* L. fruit extracts is effective against inflammation disorders and has a high concentration of phenolic content [50].

CONCLUSION

Conclusively, the present study demonstrated that AQE and EE of *Ficus carica* L. fruit exerted strong anti-inflammatory activity in λ-carrageenan-induced mice edema probably due to the presence of polyphenols inhibiting the mediators of inflammation sharing anti-inflammatory pathways with 2-[2-(2,6-dichloroanilino) phenyl]acetic acid. Thus, the *Ficus carica* L. fruit is a prime candidate to be a natural alternative in the treatment of inflammation.

Abbreviations: ANOVA, analysis of variance; **AQE**, Aqueous extract; **ATT**, Acute toxicity test; **AUG%**, percentage of augmentation of edema; **AZ**, Azendjar; **b.wt**, body weight; **CE**, Catechin equivalent; **COX**, cyclooxygenase; **CSPFE**, controlled and specific-pathogen free environment; **CTC**, Condensed tannin content; **DW**, dry weight; **EE**, ethanolic extract; **FCR**, Folin-Ciocalteu reagent; **GA**, Gallic acid; **GAE**, Gallic acid equivalent; **H&E**, Hematoxylin–eosin; **i.g.g**, intra gastric gavage; **IL**, interleukin; **INH%**, percentage of inhibition of edema; **ITAFV**, Technical Institute for Fruit Trees; **LOX**, lipoxygenase; **LSD**, least significant difference; **NSAIDs**, Non-Steroidal Anti-Inflammatory Drugs; **OECD**, Organization for Economic Co-operation and Development; **PG**, prostaglandin; **PIA**, Pasteur institute of Algeria; **QE**, Quercetin equivalent; **SAIDs**, steroidal anti-inflammatory drugs; **SD**, standard deviation; **SEM**, standard error of mean; **STD**, standard; **TFC**, Total flavonoid content; **TNF-α**, Tumor necrosis factor-α; **TPC**, Total phenolic content; **w/v**, weight/volume.

Conflict of Interest. The authors declared that there is no conflict of interest.

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