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Exploring the chemical profile, antioxidant, *in vivo* anti-inflammatory and gastroprotective properties of watermelon (*Citrullus lanatus* thunb.) in rats

Explorando el perfil químico, antioxidante, antiinflamatorio *in vivo* y propiedades gastroprotectoras de la sandía (*Citrullus lanatus* thunb.) en ratas

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ABSTRACT

Citrullus lanatus thunb. (watermelon) belonging to the Curcubitaceae family, is the most important crops worldwide. The present work aims to estimate the polyphenolic content, anti-inflammatory and antiulcer properties, in addition to the antioxidant activity of ethanolic extract of watermelon flesh fruit. The ethanolic extract of watermelon fruit contains secondary metabolites, polyphenols, flavonoids and tannins. Reducing power test and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging assay were used to measure the antioxidant activity. In the two tests that were examined, watermelon ethanolic extract demonstrated a potent antioxidant potential. Pretreatment of rats with watermelon extract at the doses of 200 and 600 mg·kg^{-1} demonstrated significant anti-inflammatory effect and decreased carrageenan induced paw edema, with inhibition percentages of 57.24 ± 3.18 and 69.00 ± 2.80%, respectively. However, the doses 50, 200, and 600 mg·kg^{-1} of watermelon extract pretreated to rats reduced gastric mucosal injury on ethanol induced acute gastric ulcer with percentages protection of 75.01 ± 0.77, 92.38 ± 2.98 and 95.01 ± 0.81% compared to omeprazole (95.92%). This study revealed that watermelon fruit consumption could be a promising anti-inflammatory and gastroprotective agents.

Key words: *Citrullus lanatus* thunb; flavonoids; inflammation; oxidative stress; polyphenols; tannins; ulcer

RESUMEN

Citrullus lanatus thunb. (sandía), perteneciente a la familia Curcubitaceae, es el cultivo más importante a nivel mundial. El presente trabajo tiene como objetivo estimar el contenido polifenólico, las propiedades antiinflamatorias y antiulcerosas, además de la actividad antioxidante del extracto etanólico de pulpa de sandía. El extracto etanólico de sandía contiene metabolitos secundarios, polifenoles, flavonoides y taninos. Se utilizaron pruebas de poder reductor y ensayo de eliminación Ácido 2,2-azino-bis(3-etil benzotiazolina-6-sulfónico) (ABTS) para medir la actividad antioxidante. En las dos pruebas examinadas, el extracto etanólico de sandía demostró un potente potencial antioxidante. El pretratamiento de ratas con extracto de sandía a las dosis de 200 y 600 mg·kg^{-1} demostró un efecto antiinflamatorio significativo y disminuyó el edema de la pata inducido por carragenina, con porcentajes de inhibición de 57,24 ± 3,18 y 69,00 ± 2,80 %, respectivamente. Sin embargo, las dosis de 50, 200 y 600 mg·kg^{-1} de extracto de sandía pretratado a ratas redujeron la lesión de la mucosa gástrica en la úlcera gástrica aguda inducida por etanol con porcentajes de protección de 75,01 ± 0,77, 92,38 ± 2,98 y 95,01 ± 0,81 % en comparación con omeprazol (95,92 %). Este estudio reveló que el consumo de sandía podría ser un prometedor agente antiinflamatorio y gastroprotector.

Palabras clave: *Citrullus lanatus* thunb; flavonoides; inflamación; estrés oxidativo; polifenoles; taninos; úlcera

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INTRODUCTION

Free radicals are byproducts of regular metabolism within cells. Reduction of pathogens, wound healing, and cell regeneration are among the physiological processes that are positively impacted by the production of a small quantity of reactive oxygen species (ROS). However, excessive ROS production upsets the body's equilibrium and causes oxidative tissue damage [1]. Oxidative stress is a state imposed by an excess of ROS or a reduction in antioxidant defense mechanisms. It is closely linked to the pathogenesis of numerous diseases as cardiovascular diseases, diabetes, atherosclerosis, inflammation, and peptic ulcer [2].

Gastric ulcer is an injury of the stomach mucosa that may be caused by an imbalance in the substances that protect the mucous (mucous-bicarbonate protective layer, prostaglandins, growth factors, cells renewal, mucosal blood flow) and the aggressive factors (acid, pepsin, free radicals) [3]. Various factors including *Helicobacter pylori* exposure, smoking, stress, alcohol consumption, poor diet, and non streroidal anti-inflammatory medication (NSAIDs) addiction are implicated in the pathophysiology of gastric ulcer [4]. Notably, proinflammatory cytokines and production of free radicals have been linked to this pathology [5]. Proton pump inhibitors, antimuscarinic drugs, H2 receptor antagonists, and acid-independent drugs which are triggered by antibiotics against *H. pylori* are the mainstays of treatment for gastric ulcers [6]. These medications may have adverse side effects such arrhythmia, hypersensitivity, and hematopoietic alterations, which would limit their effectiveness [7]. Thus, research of safe and efficient natural alternatives for gastroprotective drugs. In this regard, medicinal plants, fruits and vegetables may be considered as a proliferating source with a large range of biological activities.

Citrullus lanatus thunb. (watermelon) belonging to the Curcubitaceae family, is the most important crops worldwide. It is consumed as byproducts, such as juices, jellies, sauces, sweets and compotes with exceptional nutritional value, and is an essential fruit in the supply of bioactive chemicals in food for humans [8]. This fruit is used traditionally for its cooling, aphrodisiac, astringent, strengthening, indigestible, expectorant, diuretic properties, for blood purification, quelling of thirst, biliousness treatment, and has beneficial properties for scabies, itches, and painful ears [9]. Watermelon flesh shows various health benefits including antioxidant, antimicrobial, analgesic, laxative, antidiabetic, anti-obesity effects and contains many dietary antioxidants such as phenolics, carotenoids, and flavonoids, in addition to vitamins, mineral salts, and amino acids (arginine and citrulline). Natural substances, including ascorbic acid and lycopene, carbohydrates, cardioglycosides, terpenoids (cucurbutacine E), oils, and fats [10, 11, 12].

Nonetheless, little research is exploring the medicinal potential of *C. lanatus* flesh in Algeria, Regarding its ability to reduce inflammation and ulcers. Further, research is needed to evaluate the effective concentration for its function. Thus, this study aimed to quantify the amount of polyphenols, flavonoids, and tannins as well as the antioxidant and *in vivo* anti-inflammatory activities and gastroprotective property on ethanol-induced ulcer model in rats of the ethanolic extract of Algerian watermelon fruit, variety Crimson sweet were investigated.

MATERIALS AND METHODS

Animals

The Wistar rats *(Rattus norvegicus)* of male sex (180-200 g) (PS 600. R2, Germany) obtained from Pasteur institute in the capital Algiers. For one week of adaptation, the animals were maintained under laboratory settings (temperature of 25 ± 1°C, 55 ± 5% humidity and standard with a 12-hour daylight cycle). They were also given access to an unlimited supply of water and commercial rat food containing 21% of proteins.

Plant material and preparation of ethanolic extract

Watermelon was chopped in Setif province (Northeast Algeria) and grated after being cleaned and its flesh separated from the rind. The protocol used by Markham [13] was followed in the preparation of ethanolic extract. To maximize the extraction of phenolic chemicals, 1 kg of mixed flesh was combined with 5 L of ethanol (80%) and kept for five days at room temperature. Following the mixture's filtration with Whatman paper, the filtrates were collected and concentrated using a rotary evaporator (Buchi rotavap R-205, Switzeland) at 40°C. The crude extract was dried in the ovenat 37°C for 72 h (Memmert UM200, Germany).

Phytochemical profile

Total polyphenols content estimation

Folin Ciocalteu method was used to evaluate the content of phenolic compounds in the extract [14]. 100µL of watermelon extract and 500 µL of the diluted Folin-Ciocalteu reagent were combined. After 4 min, 400 µL of sodium bicarbonate solution (Na_2CO_3) with concentration of 7.5% was added. At 765 nm (Shimadzu UV 1800 spectrophotometer, Japan), the absorbance was measured following 90 min at dark room temperature. The total polyphenols content was determined, and the data are given as milligrams equivalent of gallic acid per gram of extract (mg GAE·g^{-1} of extract).

Flavonoids content estimation

Aluminum chloride ($AlCl_3$) technique was followed to quantify the flavonoids content [15]. Briefly, 1 mL of watermelon extract was added to 1 mL of 2% aluminium chloride solution diluted in methanol. After incubating for 10 min at 430 nm, the absorbance was determined. The flavonoids content was determined and the data were expressed as milligrams equivalent of quercetin per gram of extract (mg QE·g^{-1} of extract).

Tannins content estimation

The tannins content was estimated using the method of Gharzouli *et al.* [16]. 1 mL of cow blood with absorbance of 1.6 was combined with 1 mL of watermelon extract. After the mixture solution was left for 20 min at room temperature, it was centrifuged for 10 min at 4000 G (Sigma 3-30K, Germany), and the absorbance of the supernatant was read at 756 nm. The equivalent tannic acid in milligrams per gram of extract (mg TAE·g^{-1} of extract) was reported for the results.

Evaluation of *in vitro* antioxidant activity

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging test

Watermelon extract' free radical scavenging property was assessed using ABTS radical following the modified method of Re *et al.* [17]. To

obtain ABTS solution, a mixture of 7 mmol·L^{-1}of the aqueous ABTS and 2.45 mmol·L^{-1} of persulfate solution was diluted with methanol and incubated for 16 h. After that, 1 mL of the ABTS methanolic solution was mixed with 50 µL of watermelon extract and the absorbance was recorded at 734 nm after 15 min. Using the following formula, the ABTS free radical inhibition percentage (I%) was determined:

$$ABTS\ activity\ (\%) = \left\{\frac{Acontrol - Asample}{Acontrol}\right\} \times 100$$

Reducing power test

Reducing power of watermelon flesh ethanolic extract was estimated following the techinique of Chung *et al.* [18].The procedure involved mixing 0.1 mL of phosphate buffered saline (0.2M, pH=6.6), 0.1 mL of potassium ferricyanide (K_3FeCN_6) and 0.1 mL of watermelon extract. After the mixture was incubated for 20 min at 50°C in water bath (Memmert WNB22, Germany), 0.1 mL of 1% trichloroacetic acid was added to stop the process and the mixture was centrifuged for 10 min at 4000 G. To 0.25 of the supernatant, 0.25 mL of H_2O and 0.5 mL of 0.1% Ferric chloride ($FeCl_3$) were added. A 700 nm measurement, the absorbance was determined.

In vivo anti-inflammatory activity estimation

Paw edema induced by carrageenan in rats was used to evaluate the inflammation inhibition activity of watermelon ethanolic extract according to the method described by [19]. Rats were divided into four groups (n=5). Groups 1, 2, 3, and 4 were treated with distilled water, indomethacin as anti-inflammatory drug reference (20 mg·kg^{-1}), watermelon extract (200 and 600 mg·kg^{-1}), respectively. one hour after the pretreatment, the left subplantar hind paw was injected with 0.1 mL of carrageenan (1% in NaCl) to cause acute inflammation. Using digital calliper, measurements of edema volume were taken at 0 h before the inflammation induction (V0) and every hour until 6 h (V t). The inhibition percentages (I%) of paw edema was calculated as follows:

$$Inhibition\ (\%) = \left\{\frac{(Vt - V0)\,control - (Vt - V0)\,extract}{(Vt - V0)\,control}\right\} \times 100$$

Evaluation of antiulcer effect

The gastroprotecive potential of watermelon ethanolic extract was determined according to the method of Abdulla *et al.* [20] with slight modifications. Five groups of rats (n=5) were formed. They were allowed to consume water without restriction until 1 h before to the experiment, but they had to fast for 24 h before the beginning of experiment. Group 1 was given distilled water (5 mL·kg^{-1}) and considered as negative control; Group 2 was given proton pump inhibitor drug; omeprazole (20 mg·kg^{-1}) and considered as antiulcer group according to Sreeja *et al.* [21];Groups 3, 4,and 5 were given watermelon flesh extract (50, 200, and 600 mg·kg^{-1}). All of the rats' groups were given an oral dose of absolute ethanol (2.5 mL·kg^{-1}) 1 h following the extract pretreatment. After the administration of absolute ethanol for 30 min following Mamache *et al.* [22],the animals were killed with cervical dislocation and their stomachs were taken out, opened, washed with NaCl (0.9%), and then were taken pictures. The total area of the injuries was calculated using image J software (Wayne Rasband, NIH, USA).The percentages of ulceration (Ulcer index) and the preventive index (PI) were calculated using the following formula:

$$Ulceration\ (\%) = \left\{\frac{total\ ulceration\ area}{total\ mucosa\ area}\right\} \times 100$$

$$PI\ (\%) = \left\{\frac{Uc - Ut}{SUc}\right\} \times 100$$

Statistical analysis

Graphpad Prism version 5.00 was used to analyze all the data. Following one-way ANOVA to ascertain group differences, Dunnet's test was conducted in all experiments. The findings presented *in vitro* and *in vivo* were expressed as mean ± standard error of mean (SEM) and mean ± standard deviation (SD), respectively. When P<0.05, the values were taken to be significantly different.

RESULTS AND DISCUSSIONS

Polyphenols, flavonoids, and tannins contents

The quantification of total polyphenols and flavonoids contents revealed that watermelon flesh extract contains amount of 37.01 ± 2.84 mg GAE·g^{-1} of extract and 0.69 ± 0.08 mg QE mg·g^{-1} of extract, respectively. However, the estimated tannins concentration was 49.77 mg TAE.g^{-1} of extract. Phenolic content of *C. lanatus* extract was superior to that found in a Ghanian study of Neglo *et al.* [23] in which methanolic fraction contained 0.010 ± 0.001 mg·g^{-1} of extract. The fresh juice of watermelon was found to contain 17-20 mg of polyphenols per 100 mL. Also, hydrolysable phenols, which make up 63% of all phenolic compounds, were found in high concentrations in watermelon peels according to phytochemical analyses of Pérez-Jiménez and Saura-Calixto [24]. This fraction mainly contained flavanols and the acids hydroxybenzoic and hydroxycinnamic with concentration of 1.32 g·g^{-1}of dry weight. The secondary metabolites have been found to have a large variety of biological activities as analgesic, anticancer, anti-inflammatory, and antibacterial properties, in addition to having strong antioxidant activity [25].

Antioxidant activity estimation

ABTS radical scavenging effect

ABTS test is the common spectrophotometric procedure, and it depends on the ABTS radical's capacity to undergo decolorization in the addition of phytochemical antioxidants by either receiving an electron or donating proton [26]. As seen in TABLE I, the ABTS radical scavenging effect of *C. lanatus* extract significantly (P<0.001) was lower with inhibition concentration(IC_{50}) of 0.468 ± 0.05 mg·mL^{-1} than that of butylated hydroxytoluene (BHT) (IC50 = 0.015 ± 0.0003 mg·mL^{-1}). This free radical scavenging effect may be due to the phenolic content, as demonstrated by its capacity to extract a significant quantity of flavonoids and polyphenols, which are in charge of many plant species' antioxidant properties [27].Radical scavenging is the main way that polyphenols exert their antioxidant action [28]. Single electron transfer or hydrogen atom donation can be used to achieve it. Furthermore, the antioxidant activity of phenolic molecule is influenced by factors as number and location of OH groups inside it [29].The variability in antioxidant activity with respect to phenolic concentration can be explained by several factors. The region in which food plants were grown, altitude, environmental factors like irrigation, soil, light, temperature, harvested season, exposure pathologies and pests, industrial processing, drying, storage, and extraction technique

are some of the factors that may affect the antioxidant potential and polyphenolic content of food plants [30, 31].

Ferric reducing power

The strong absorbance of Prussian blue complex may be used to measure the reducing power after the reduction of ferricyanide to ferrocyanide in the mixture solution [30].

This result indicated that the ferric reducing power of *C. lanatus* ethanolic extract and the positive standard (BHT) increase with their concentration. Higher ferric reducing power was demonstrated by increased of the absorbance reaction mixture. It was found that the ferric reducing power of *C. lanatus* extract was moderate with effective concentration (EC_{50}) of 3.58 ± 0.04 mg·mL^{-1} compared to BHT as reference standard (EC_{50} = 0.007 ± 0.002 mg·mL^{1}) (TABLE I) and the difference was significant (P<0.001). It can be seen that watermelon flesh extract has a potent reducing ability which may be due to the presence of reductants functioning as electron donors [32]. In this sense, polyphenols are well recognized to be good electron donors [33], which allowing them to function as antioxidants that break the chain and scavenge free radicals. These results suggest that watermelon extract could be able to donate electrons to scavenge reactive oxygen species. Antioxidant chemicals that function as reductants stop the radical chain reaction functioning as electron donors by giving a hydrogen atom to the metal complex [34].

TABLE I
In vitro antioxidant activity

	ABTS IC_{50} (mg·mL^{-1})	Ferric reducing power EC_{50}(mg·mL^{-1})
Citrullus lanatus extract	0.468 ± 0.05 ***	3.58 ± 0.04 ***
BHT	0.015 ± 0.0003	0.007 ± 0.002

Comparison was done with BHT as positive standard ; ***P<0.001

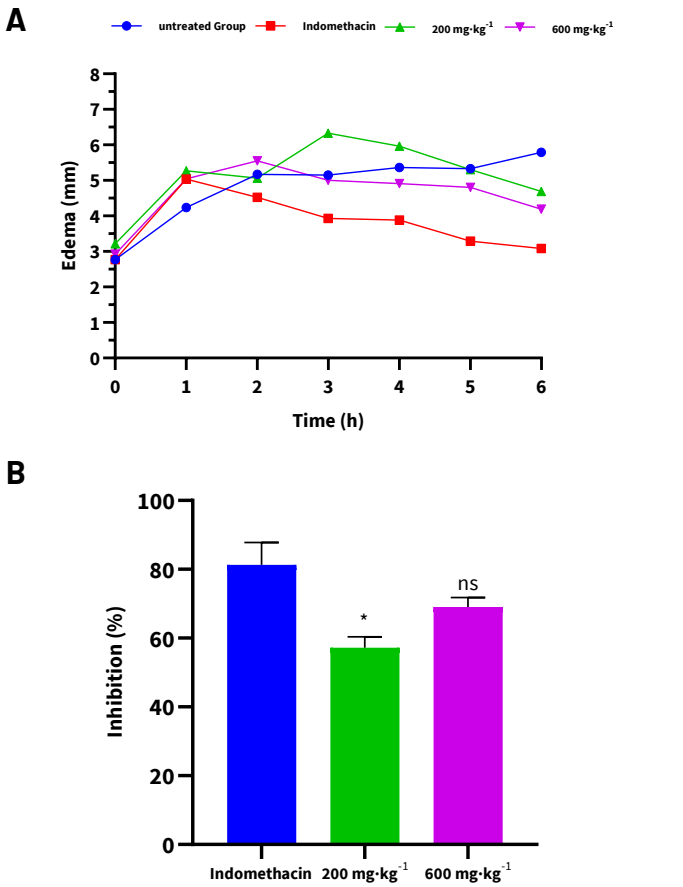

FIGURE 1. Anti-inflammatory effect of watermelon extract on carrageenan induced paw edema in rats. A: Paw edema volume (mm). B: % inhibition of edema volume. The results are presented as mean (%) ± SEM (n=5). ns: no significant,*: significantly different at P<0.05 vs indomethacin

In vivo anti-inflammatory activity

The carrageenan induced paw edema technique was used to assess the acute anti-inflammatory property of the watermelon ethanolic extract [19]. The thikness of rat's paw was measured within 6 h after the injection of carrageenan. As shown in FIG. 1.A, the pretreatment with *C. lanatus* ethanolic extract (200 and 600 mg·kg^{-1}) significantly decreased the edema volume induced by carrageenan within 3 h when compared with the NSAIDs medication, indomethacin (20 mg·kg^{-1}). Injection of carrageenan into the hind paw induced an increase in edema reaching its maximum at 6 h with edema thickness of the control group was 5.78 ± 0.80 mm. This edema was reduced by indomethacin to 3.08 ± 0.06 mm and reduced by the two doses of *C. lanatus* extract to 4.68 ± 0.74 and 4.19 ± 0.85 mm, respectively. These last two doses of watermelon extract presented an inhibition percentages of 57.24 ± 3.18 and 69.00 ± 2.80%, respectively (FIG. 1.B). The effect of the 600 mg·kg^{-1}dose was similar to that of indomethacin (I% = 81.30 ± 6.54%).

Sulphated sugars found in carrageenan cause biphasic reactions involving the activation of the complement system and inflammatory mediators [35]. Histamine, serotonin, and kinins mediate the inflammatory impact in the first and second hours, whereas prostaglandin synthesis and cyclooxygenase 2 activation mediate the inflammatory effect in the subsequent phase, which lasts from 3 to 6 h [36]. Thus, the phenolics present in the ethanolic extract of *C. lanatus* flesh may be principally responsible for any potential anti-inflammatory properties. The explicative and strong mechanism of flavonoids' anti-inflammatory action may be their inhibition of proinflammatory mediators. This is also consistent with several studies that claim the potent anti-inflammatory properties of different plants that include chemical components like polyphenols, which function by blocking prostaglandin pathways [37]. Furthermore, it was shown the compound Cucurbitacin E, which was extracted from *C. lanatus* var. *citroids*, inhibits reactive oxygen species (RNS), cyclooxygenase (COX), and the synthesis of nitric oxide in macrophages activated by lipopolysaccharids and interferon γ (IFNγ) [38].

Consuming watermelon and L-arginine which is one of the constituents of watermelon increased the liver's expression of endothelial nitric oxide while that of fatty acid synthase, cyclooxygenase 2 was decreased. The findings are consistent with the theory that arginine and watermelon intake modify pertinent gene expression to enhance cardiovascular disease risk variables such as inflammation, antioxidant capacity, and lipid profile [39].

Gastroprotective activity of watermelon extract

Macroscopic examination

Rats pretreated with absolute ethanol displayed a wide range of macroscopic lesions (FIG. 2A) and severe hemorrhagic lineage, varying in size and scattered throughout the glandular portion of the stomachs, suggesting the formation of a fully developed gastric ulcer. In comparison to the ethanol treated control, pretreatment with watermelon extract (50, 200, and 600 mg·kg^{-1}) reduced the damage to the gastric mucosa dose dependently (FIG .2B, C, D). Additionally, the stomach mucosal folds flattened and the regions of gastric ulceration were lessened in the rats given omeprazole (20 mg·kg^{-1}) prior to ethanolic induction (FIG. 2E).

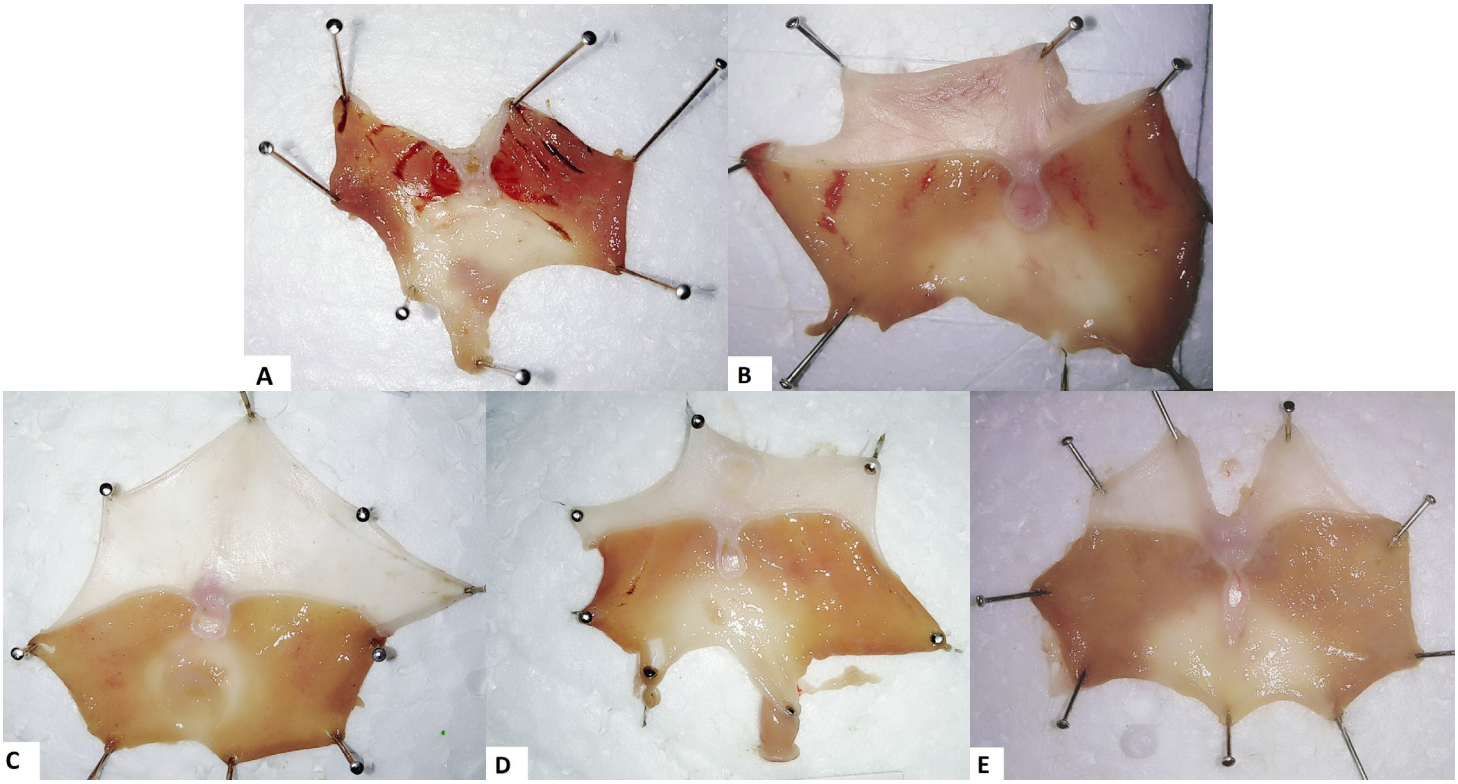

FIGURE 2. Effect of pretreatment with watermelon extract on the gross appearance of the gastric mucosa in ethanol induced ulceration. (A): Absolute ethanol, (B): watermelon extract (50 mg·kg^{-1}), (C): watermelon extract (200 mg·kg^{-1}), (D): watermelon extract (600 mg·kg^{-1}), (E): omeprazole (20 mg·kg^{-1})

Evaluation of antiulcerative activity

As shown in FIG. 3, administration of ethanol caused severe gastric lesions with a UI value of 31.96 ± 2.41% in ethanol treated group. While rats treated with *C. lanatus* extract at 50, 200, and 600 mg·kg^{-1} doses on gastric mucosal injury induced by ethanol exhibited a statistically a significant decrease in the number and the severity of gastric injuries. The protective indexes of *C. lanatus* extract were 75.01 ± 0.77, 92.38 ± 2.98 and 95.01 ± 0.81%. These values were compared to omeprazole with protective index of 95.92 ± 1.98 (FIG. 4).

In this work, the results agree with those of Shama *et al.* [40], who claimed that the aqueous extract of *C. lanatus* var *citroides* flesh exerted a protective effect on pyloric ligation and indomethacin induced ulceration. The antiulcer effect of *C. lanatus* extract could be attributed to its capacity to scavenge free radicals, reduce acid secretory parameters, and enhance the gastric mucosal barrier. The presence of bioactive components in the extracts may increase protective factors, preventing damage to the gastric mucosa and maintaining its integrity [41].

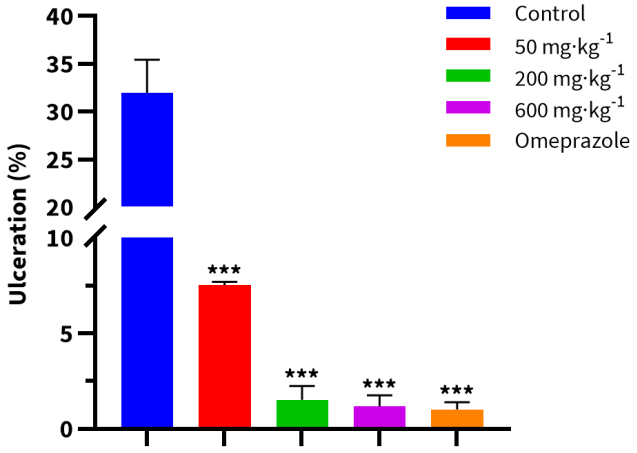

FIGURE 3. Effect of pretreatment with watermelon extract on ethanol induced gastric lesions. Ulceration percentage. The results are represented as mean % ± SEM (n=5). ns: no significant, *: significantly different at *P*<0.001 vs control or omeprazole**

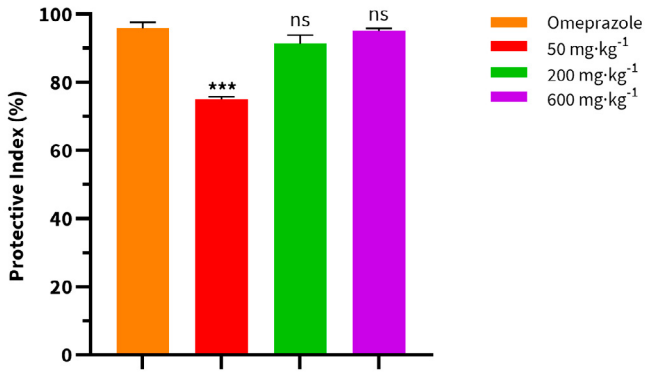

FIGURE 4. Effect of pretreatment with watermelon extract on ethanol induced gastric lesions. Protective index expressed in %. The results are represented as mean % ± SEM (n=5). ns: no significant, *: significantly different at *P*<0.001 vs control or omeprazole**

Previous research has indicated that active principle with antiulcer properties include flavonoids, alkaloids, tannins, soponins, and terpenoids [42]. Flavonoids are polyphenolic compounds that stimulate the release of stomach mucus and are also known to have antioxidant properties. Tannins are known to "tar" the stomach mucosa's outermost layer, making it less permeable and more resistant to irritants and chemicals as well as mechanical harm [43]. According to this study, *C. lanatus* may have anti-inflammatory activity that helps in the prevention of mucosa from lesions.

CONCLUSIONS

This study showed that flesh ethanolic extract from crimson sweet, a variety of watermelon largely consumed in Algeria contains secondary metabolites; polyphenols, flavonoids , and tannins and demonstrated strong radical scavenging and reducing power. Moreover, the ethanolic extract of watermelon flesh demonstrated potent anti-inflammatory and antiulcer properties. These findings suggest that watermelon consumption could be useful alternative treatment for the management of inflammatory and ulcer diseases.

Conflict of interest

The contributors attest that there is no conflicts of interest.

Ethical approvals

The European Union Guidelines with Number of 2010/63/Eu were followed in this study for all experiments and authorized by the Algerian Association of Sciences committee under number law 88-08 of 1988.

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