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Article

Protein and sugar contents, total antioxidant capacity, analgesic and antiulcer activities of quince fruit extract

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ABSTRACT

Cydonia oblonga belongs to the *Rosaceae* family, known in Algeria as sfarjel. It is a good source of secondary metabolites with antidiabetic, antihemolytic and antiallergic effects. The present study was undertaken to estimate total proteins and sugar contents and *in vitro antioxidant, analgesic,* and gastroprotective activities of quince fruit ethanolic extract (QFEE). Proteins and sugar contents of QFEE were determined to be 0.06 ± 0.002 mg BSA E/ g of dry extract and 111.95±0.02 mg GE/g of dry extract, respectively. Using total antioxidant capacity (TAC), QFEE demonstrated a critical antioxidant activity with an IC_{50} value of 0.39±0.008 mg/ml. Oral administration of QFEE at 200 and 600 mg/kg doses to rats gave a dose-dependent gastroprotective effect in an ethanol model-induced ulcer, with protection percentages of 77.75 and 91.81 %, respectively. The same doses of extract had analgesic activities against acetic acid-induced abdominal contraction. According to these findings, quince extract is an essential source of antioxidant compounds that may have analgesic properties and shield the stomach from developing ulcers.

Keywords: *Cydonia oblonga* Mill, Sugar content, protein content, Antioxidant activity, Analgesic, Ulcer.

INTRODUCTION

Among the most common diseases of the digestive system is the gastric ulcer. This disease's multifactorial pathophysiology is brought on by an imbalance between the factors that protect and destroy the stomach mucosa (pepsin and acid) and is aggravated by infection, smoking, stress, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs), and excessive alcohol consumption [1]. Various factors, including the interaction between leukocytes and endothelium, neutrophil infiltration, cytokine imbalance, and oxidative mucosal stress, cause NSAID-induced gastric mucosal injury. Mucosal damage is caused by increased reactive oxygen free radical production, which cytotoxically affects epithelial cells and increases the mucosa's susceptibility to damage [2].

Consuming food rich in antioxidant compounds can reduce the body's naturally occurring free radicals, protecting against the risk of different diseases [3]. It has been found that eating fruits with a significant antioxidant capacity is more effective than those with low antioxidant capacity in reducing oxidative damage associated with the aging process and oxidative stress-related diseases [4].

Cydonia oblonga fruit (*Rosaceae*), cultivated in Algeria, is more nutritious than many other fruits. It contains many compounds: organic acids, tannins, proteins, sugars, minerals, vitamins, and vitamin C. It is also a good source of flavonoids and phenolic acids, which have antimicrobial, anti-carcinogenic, anti-inflammatory, anti-ulcerative, and antiallergic qualities [5]. For this reason, the present study aims to determine sugar and protein contents and evaluate the antioxidant, anti-inflammatory and gastroprotective effects of ethanolic extract prepared from quince fruits.

MATERIALS AND METHODS

Plant extract

Fruits of *Cydonia oblonga* were collected in September from the Setif region (East of Algeria). They were peeled and cleaned, and the seeds were removed.

Animals

Male Swiss Mice (26–30 g) and Albinos Wistar rats (180–220 g) were obtained from Pasteur Institute, Algiers, Algeria, Algiers, and were kept in polypropylene cages maintained under standard laboratory conditions. Animals were given a week's acclimatization period and had free access to food and water. All experimental studies were approved by the Committee of the "Algerian Association of Sciences in Animal Experimentation" (http://aasea.asso.dz/ articles/) under law No. 88-08/1988, associated with veterinary medical activities and animal health protection (N° JORA: 004/1988).

Preparation of fruit extract

One kg of ground pulp fruits was mixed with 5 L of ethanol diluted (20%) for five days at room temperature [6]. The stained mixture was filtered, and the rotavapor instrument eliminated the ethanol to give ethanolic quince fruit extract(QFEE).

Phytochemical analysis

Determination of total sugar content

To determine the sugar content in the extract, the dosage was carried out according to the method of Dubois et al. [7]. Briefly, 1 ml of extract and 1 ml of phenol (5%) were mixed. After shaking, 5 ml of concentrated sulfuric acid was added, and the absorbance was measured at 490 nm. The results were expressed in milligrams of glucose equivalents per gram of dry extract (mg GE/ g of extract)

Determination of total protein content

The Bradford method is a colorimetric assay that manifests the change in the color of Coomassie blue[8]. This method is rapid and sensitive (between 0.2 and 20 µg proteins). 0.004% Coomassie blue was dissolved in 4% ethanol and 10% phosphoric acid to create the reagent. After mixing the diluted reagent with 100 µl of extract, the absorbance at 595 nm was measured against a blank. The results were represented as milligrams of Bovine serum albumin equivalents per gram of dry extract (mg BSA E/ g of extract).

In vitro antioxidant activity using total antioxidant capacity (TAC)

The antioxidant capacity of the extract was evaluated according to the procedure of Shirwaikar et al.[9]. 0.1 ml of extract was combined in an Eppendorf tube with 1 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a bathroom at

95°C for 90 minutes. After cooling to room temperature, the absorbance of the aqueous solution of each tube was measured at 695 nm against blank.

Analgesic activity

The analgesic effect of quince fruit extract was investigated, following the method of Ishola et al. [10]. Female mice were divided into four groups, each group containing six mice (n = 6). The first group (positive control) was treated orally with aspirin, which is used as a reference drug at a concentration of 100 mg/kg. The second group (negative control) was treated orally with distilled water. Both groups (third and fourth groups) were treated orally with quince extract at 200 mg/kg and 600 mg/kg, respectively. After 60 minutes, acetic acid was injected into the peritoneum at a concentration of 0.1%. Five minutes after the injection, the number of abdominal contractions is counted for twenty-five minutes. This equation calculates the analgesic effect: (%) =(Cn-Ct/Cn)*100.

Cn is the mean number of contractions of the control group. Ct is the mean number of contractions for the treated group.

Antiulcer activity

The gastric protective effect of quince ethanolic extract was evaluated using an ethanol-induced gastric ulceration model in rats [11]. The experimental animals were fasted for 24 hours with free access to water until 1 hour before the experiment. Rats were divided into four groups; each group consisted of five rats as follows: the first group, which represented the negative control, received orally 1 ml of distilled water; the second group, which represented the positive control, was treated with 1 ml of Omeprazole (20 mg/kg), which was approved as an antiulcer agent. The third and fourth groups were treated with two doses of QFEE(200 mg/kg and 600 mg/kg, respectively).

After an hour of treatment, each group received 0.5 ml of ethanol, and after 30 min, rats of all groups were killed by cervical dislocation. Rats were dissected, and stomachs were removed and opened according to the large curve. Then, they were cleaned well with distilled water, fixed, and photographed to calculate the area of ulcers using Image J. The following formula calculated the gastric protective effect:

The protection % = the rate of ulceration of the control - the rate of ulceration of the treatment / the rate of ulceration of the control x100.

Statistical analysis

Statistical tests were carried out using Graph Pad Prism (Version 7.00). *In-vitro* results were expressed as mean ± standard deviation (SD) and *in-vivo* results as mean ± standard error of means (SEM). Results were analyzed for significance using one-way analysis of variance (ANOVA) followed by Dunnet's 5% probability level test.

RESULTS

Total sugars and protein contents in extract

The total sugars and protein contents in the ethanolic extract of quince fruit are shown in Table 1. Bradford is a rapid and precise method for estimating protein content [12]. Total sugars and their derivatives react with

sulfuric acid and phenol to produce an orange-yellow color [13]. The results were expressed as mg BSA E per g of dry extract and mg GE per g of dry extract, respectively. The quince extract was rich in sugars with a value of 111.95±0.02 mg GE per g of dry extract, while the amount of proteins was low with a value of 0.06±0.002 mg BSA E per g of dry extract.

Title 1	Total sugar content mg GE/g	Total protein content mg BSA E/g
QFEE	111.95±0.02	0.06±0.002

Results are expressed as mean ± sd (n=3).

Table 1. Total sugars and proteins contents in *C. oblonga* extract.

Antioxidant activity using TAC of *C. oblonga* fruit extract

The total antioxidant capacity revealed that the QFEE exhibited a low antioxidant activity (IC_{50}=0.395±0.0008 mg/ml, $p < 0.001$), which is lower than vitamin C used as standard (IC_{50} = 0.0058 ± 9.10 $^{-5}$ mg /ml (Table 2).

Title 1	QFEE	Vit C
IC_{50} (mg/ml)	0.39 ±0.0008***	0.0058±9.10^{-5}

Data are expressed as mean±SD (n=3). Vit C was used as a positive standard. ***p <0.001

Table 2. *The QFEE's in vitro antioxidant activity* was measured using the TAC test.

Analgesic activity

In this study, fruit extract at 200 and 600 mg/kg doses demonstrated a dose-dependent peripheral analgesic effect compared to the negative control (Figure 1). Both groups of the extract reduced the number of writhing, with a significant inhibition found at the highest dose (600 mg/kg) with a percentage inhibition of 60.07 %. The level of writhing reduction in the different extract doses was lower than the standard drug aspirin (100 mg/kg) (85.12%).

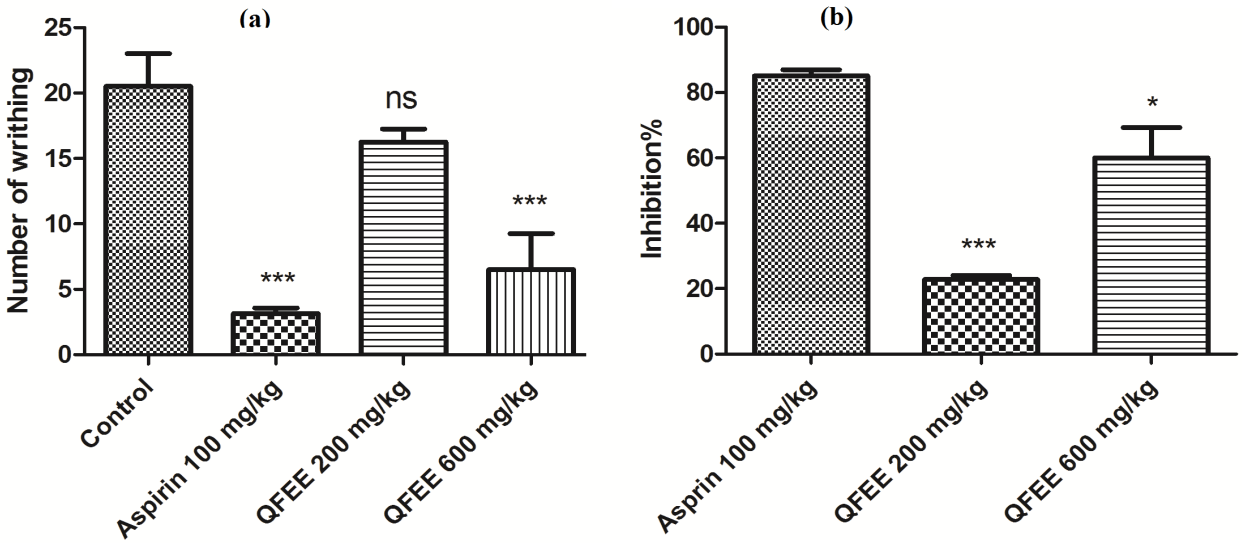

Figure 1. Effect of QFEE on pain induced by acetic acid in rat. (a), number of writhings (The comparison is made with the negative control group). (b), inhibition % (The comparison is made with the Aspirin control group). Results are expressed as means±SEM (n=5). ns: no significant, * p< 0.05, ***: P<0.001.

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Effect of QFEE on ethanol-induced gastric lesions

Macroscopic evaluation of lesions

The oral administration of absolute ethanol at a 2.5 ml/kg dose induced gastric mucosa lesions (Figure 2). Pretreatment with the QFEE decreased the number and area of gastric lesions dose-dependently. The dose of 600 mg/kg had a similar inhibition on the number of gastric lesions as a positive control omeprazole at 20 mg/kg.

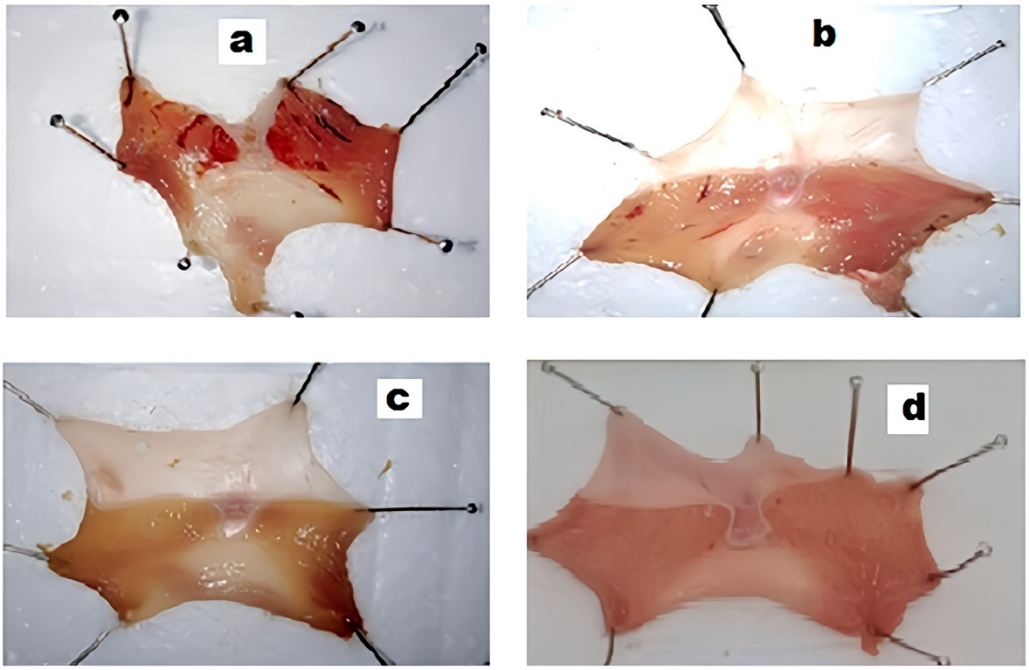

Figure 2. Effect of QFEE on ulcer gastric induced by ethanol in the rat. A, ethanol-treated control; b: quince fruit extract treated rat (200 mg/kg); c: quince fruit extract treated rat (600 mg/kg); d, Omeprazole treated rat (20 mg/kg).

Estimation of gastric protection activity

The treatment of rats with QFEE (200 mg/kg and 600 mg/kg) significantly reduced lesion area with a percentage of ulceration of 7.74±1.533 % and 2.616±0.8354%, respectively, compared to the control group (31.96±2.41%) (Figure 3). The positive control treated with Omeprazole (20 mg/kg) exhibited significant inhibition of gastric lesions (1.98±0.63%, $p \leq 0.001$). Rats pretreated with 200 mg/kg of QFEE showed a percentage protection of 77.75 ± 4.78 %, while rats given 600 mg/kg of QFEE had good protection with a value of 91.81 ±2.16%, which was comparable to the protection ratio of Omeprazole (95.92 ±1.98%) (Figure 3).

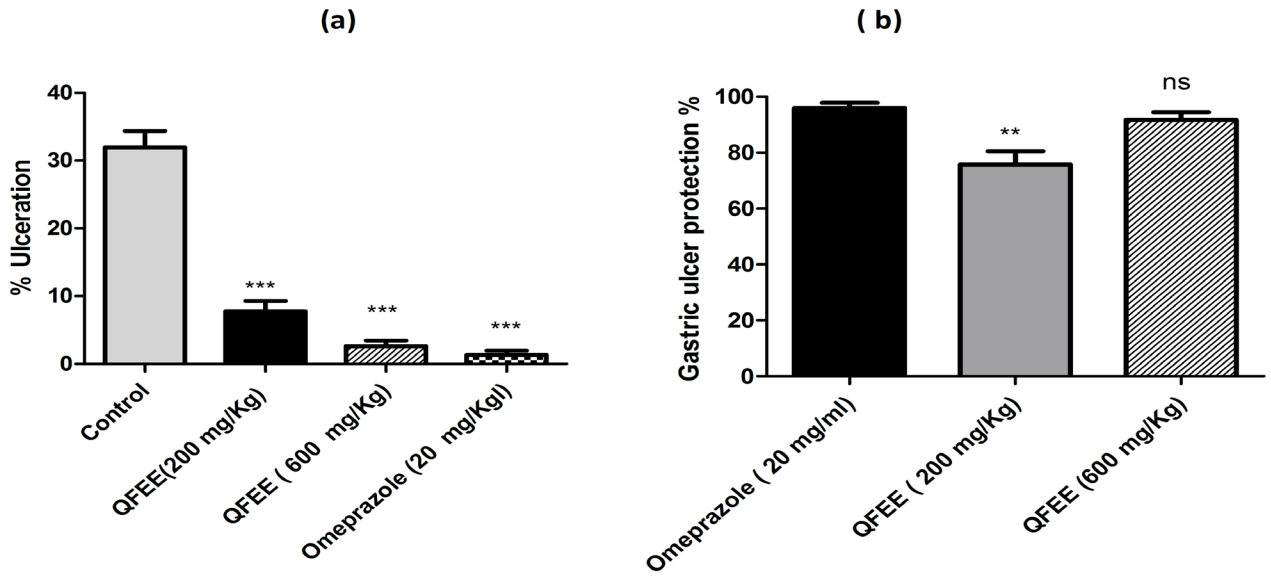

Figure 3. The effect of QFEE on gastric lesions induced by ethanol in rats. (a), ulceration expressed in %. (b), Gastroprotective effect in %. Results are expressed as means±SEM (n=5). ns: no significant, ** p< 0.01, ***: P<0.001.

DISCUSSION

Sugars act as a crucial signaling or regulatory molecule in plants, influencing the expression of genes linked to growth, development, metabolism and disease resistance. Reducing and nonreducing sugars are essential to the central metabolite pathways and assist in synthesizing secondary metabolites that improve the therapeutic effects of plants [14]. Moreover, growing evidence shows that sugars play an antioxidant role because they can scavenge reactive oxygen species (ROS). Carbohydrates may consequently be seen as fundamental compounds of a complete cellular redox system, including enzymatic scavengers like superoxide dismutase, ascorbate peroxidase and glutathione peroxidase as well as non–enzymatic metabolites like ascorbic acid, glutathione and phenolic compounds[15]. The present study showed that the *C. oblonga* ethanolic extract is rich in sugars. These results are confirmed by many studies that found that the quince fruit contains glucose, fructose, maltose, and sucrose with levels of quinic and malic acids [16-17].

Proteins are necessary for human growth. They can be obtained from either plants or animals. Our study showed that quince fruit extract is a source of proteins. The presence of these molecules improves the nutritional value of foods and helps to elevate the sensory and organoleptic properties of fruits and vegetables[18].

The total antioxidant capacity of the extract was determined using the phospho molybdenum method, which is based on the antioxidant action of the extract reducing molybdenum (VI) to molybdenum (V) and the subsequent formation of a green phosphate Mo (V) complex at acid pH of the medium with maximum absorbance at 695 nm [19]. Because of its hydrogen and electron donating ability, the phospho molybdenum test detects antioxidants such as carotenoids, ascorbic acid, tocopherol, certain phenolics, cysteine, and aromatic amines. Phytochemical research revealed the existence of numerous bioactive phytochemicals, which may be attributed to *C. oblonga's* antioxidant ability. Our results are consistent with the findings of Herrera-Rocha et

al.[20,] who found that the phenolic acids, flavonols, flavones, dihydrochalcones, and other polyphenols in 85% aqueous acetone extract of quince fruit were responsible for the considerable antioxidant activity. This antioxidant impact was more related to the synergistic effect of the variety of polyphenol components than their higher concentrations.

The acetic acid-induced writhing test is a well-recommended model for screening the peripheral analgesic potentials of natural compounds due to its sensitivity and capacity to identify antinociceptive effects of natural products [21]. Acetic acid intraperitoneal injection produces irritation and stimulation of the peritoneal cavity, creating and releasing several endogenous inflammatory mediators such as histamine, serotonin, bradykinin substance P, and Prostaglandins[22]. The possible mechanism of QFEE for producing peripheral analgesia in this model could be associated with inhibiting the synthesis and release of various endogenous inflammatory mediators and suppressing the sensitivity of peripheral nociceptors in peritoneal free nerve endings to chemical-induced pain. So, any agent that decreases the number of writhing will demonstrate analgesia by inhibiting the synthesis and release of Prostaglandins and by inhibiting the peripheral pain transmission [23].
Ulcer disease is caused by an imbalance between protective effect factors (blood flow, bicarbonate secretion, prostaglandin, antioxidant enzymes) and aggressive factors (acid and pepsin) [24]. Many factors may increase the incidence of peptic ulcer disease, including alcohol consumption, extensive use of non-steroidal anti-inflammatories, stress life and bad dietary[25-26]. In our study, an ethanol-induced gastric ulcer model was used. Ethanol administration leads to gastric mucosal damage by forming hemorrhagic lines in the glandular part of the stomach. In the present study, QFEE at 200 and 600 mg/kg doses significantly reduced lesions' size, number and surface area in gastric ulcers induced by ethanol. The QFEE at 600 mg/kg dose was also shown to have an equal protective effect, with Omeprazole used as a positive standard. Several studies have revealed the ability of quince extracts to protect the stomach from ulcers. Results of [27] confirmed the gastroprotective effect of hydroalcoholic and aqueous extracts from quince fruits in indomethacin gastric ulcers. The study of Hamauzu[28] revealed the gastroprotective effectiveness of Chinese quince in the ethanol-HCL gastric ulcer model in rats. In this published data, quince extract is rich in polyphenols and tannins, which could bind pepsin and mucosal proteins to protect the gastric wall against acid and pepsin damage. The high antiulcerogenic activity of tannins purified from Quercus species on ethanol-induced gastric lesions in mice can be responsible for inhibiting acid secretion[29].
On the other hand, the gastroprotective effect of quince fruit extract can be attributed to the presence of antioxidant compounds where mucosal damage in the stomach is associated with the generation of reactive oxygen species that exhibit a vital role in the production of lipid peroxides, leading to the inhibition of antioxidant enzymes[30]. As shown by Daniela et al. [31], the quince extract is rich in antioxidants containing flavonoids and phenolic derivates that might be considered a complementary medicine for peptic ulcers. It has been shown that the antioxidant activity is related to the anti-ulcerative effect because the free radicals produced from the neutrophil cells are an essential source of gastric damage [32]. Also, the antiulcer activity of QFEE can be related to its sugar content. The sugars produced in the leaves are polymerized during photosynthesis and then stored as starch in the fruit. The fruit's starch content increases in ripening. Between 4.3 and 7.3 g 100g-1 of starch and 9.5 to 11.1 g 100g-1 of total sugars were found in the pulp from quince strains [33]. The volume of gastric juice decreased significantly when starch plant extracts were used. A good anti-secretory effect was produced by starch. It can act on different mechanisms to reduce ulcers. The effect is mediated directly by muscarinic receptor inhibition, indirectly through ganglion blocking, or directly through secretory cell action. Additionally, the observed effects of fruit rich in sugar may result from increased enterogastrone function or inhibition of histamine or gastrin [34].

CONCLUSIONS

The extract was found to be high in sugars and contain some protein. While its antioxidant capacity was moderate, it displayed both analgesic and gastroprotective properties. The TAC assay *in vitro* antioxidant activity demonstrated its ability to convert molybdenum (VI) to molybdenum (V). Additionally, QFEE exhibited dose-dependent analgesic and gastroprotective properties. Quince extract with high content could be a source of bioactive substances employed in various biological processes.

The analgesic effect increased with dosage, suggesting potential pain relief. Additionally, the extract was significantly protected against gastric lesions in an ethanol-induced ulcer model, performing comparably to standard medication. These findings suggest that quince extract could be a natural pain reliever and hold promise for future development of dietary supplements or complementary therapies for gastric ulcers.

Author contributions

Conceptualization, S.Djidel and A.Bouaziz; methodology, S.Djidel, A.Bouaziz, N.Barghout, A.Bentahar; software, S.Djidel; validation, A.bouaziz.; formal analysis, A.Bentahar and N.Barghout; investigation, S.Djidel; resources, S.Dahamna; data curation, A.Bouaziz; writing-original draft preparation, S.Djidel; writing-review and editing, S.Djidel and A.Bouaziz, visualization, S.djidel; supervision, S.Dahamna; project administration, S.Kennouf. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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Additional information
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