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PHARMACOGNOSIE

Study of Antioxidant, Anti-inflammatory, Antinociceptive Activities and Toxicity of Stigmata of *Zea mays* Extracts

Étude des activités antioxydantes, anti-inflammatoires, antiantinociceptives et de la toxicité d'extraits de stigmates de *Zea mays*

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Abstract This study aims to investigate phytochemical screening, total polyphenol and flavonoids content, antioxidant activities and to examine toxicity, anti-inflammatory and antinociceptive effects of different extracts of stigmata of *Zea mays* from Morocco. The flavonoids and total phenols content were performed for both extracts. The aqueous and hydro-ethanolic extracts were screened for their possible antioxidant activities by three test systems, namely DPPH free radical-scavenging, reducing power and molybdenum system. Oral and Sub-acute toxicity of the hydro-ethanolic extract was evaluated in vivo. Anti-inflammatory activity of the hydro-ethanolic extract was evaluated by Carrageenan-induced rat paw edema method. The antinociceptive effect was tested by using the formalin test. Phytochemical screening of the extracts revealed a presence of flavonoids, leucoanthocyans, heterosid sterodic, coumarins, alkaloids, cardiac glycosides, anthocyanins and tannins. The flavonoids and total phenols content show higher content of flavonoids and total phenols in the hydro-ethanolic extract. It showed better antioxidant activity than the aqueous extract in the three methods used. Furthermore, the hydro-ethanolic extract with a concentration of 250 and 500 mg/kg body weight inhibited the inflammation induced by carrageenan in rats at 66.67% and 86.67% compared to 60.8% for indomethacin at 10 mg/kg after 5 h of inflammation induction. However, at dose of 500 mg/kg extract showed a pro-inflammatory effect. In the formalin test, the tolerance time of the rats was significantly higher compared to the control group. These initial results tend to support the traditional use in the treatment of cystitis, oliguria, nephritis, renal lithiasis edema, albuminuria, heart disease, slimming cures of stigmata of *Zea mays* in Morocco.

Keywords Stigmata of *Zea mays* · Total phenol content · Flavonoids content · Antioxidant activity · Toxicity · Anti-inflammatory activity · Antinociceptive activity

Résumé Cette étude a pour objectif d'étudier le criblage phytochimique, la teneur totale en polyphénols et en flavonoïdes, les activités antioxydantes et d'examiner la toxicité, les effets anti-inflammatoires et antinociceptifs de différents extraits de stigmates de *Zea mays* du Maroc. La teneur en flavonoïdes et en phénols totaux a été réalisée pour les deux extraits. Les extraits aqueux et hydroéthanoliques ont été criblés en fonction de leurs activités antioxydantes potentielles par trois systèmes de test, à savoir le piégeage des radicaux libres par le DPPH, le pouvoir réducteur et le système au molybdène. La toxicité orale et subaiguë de l'extrait hydroéthanolique a été évaluée in vivo. L'activité anti-inflammatoire de l'extrait hydroéthanolique a été évaluée par la méthode de l'œdème de la patte de rat induite par le carraghénine. L'effet antinociceptif a été testé en utilisant le test du formol. Le dépistage phytochimique des extraits a révélé la présence de flavonoïdes, de leucoanthocyanes, d'hétérosides stérodiques, de coumarines, d'alcaloïdes, de glycosides cardiaques, d'anthocyanines et de tanins. La teneur en flavonoïdes et en phénols totaux montre une teneur plus élevée en flavonoïdes et en phénols totaux dans l'extrait hydroéthanolique. Il a été montré que l'extrait aqueux dans les trois méthodes utilisées a une meilleure activité antioxydante. De plus, l'extrait hydroéthanolique à une concentration de 250 et 500 mg/kg de poids corporel inhibait

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l'inflammation induite par la carraghénine chez le rat à 66,67 et 86,67 % contre 60,8 % pour l'indométacine à 10 mg/kg après cinq heures d'induction de l'inflammation. Cependant à la dose de 500 mg/kg, l'extrait a montré un effet pro-inflammatoire. Dans le test au formol, le temps de tolérance des rats était significativement supérieur à celui du groupe témoin. Ces premiers résultats tendent à soutenir l'utilisation traditionnelle dans le traitement de la cystite, de l'oligurie, de la néphrite, de l'œdème de la lithiase rénale, de l'albuminurie, des maladies cardiaques, du traitement amincissant des stigmates de *Zea mays* au Maroc.

Mots clés Stigmate de *Zea mays* · Teneur en phénols totaux · Teneur en flavonoïdes · Activité antioxydante · Toxicité · Activité anti-inflammatoire · Activité antinociceptive

Introduction

Since the earliest times, man has used the plants, first for food, as supplements to improve the flavor of food and then for healing. Nowadays there is an immense renewal of interest by the pharmaceutical and food industries in medicinal plants and natural products for their richness in active molecules and their prospective use for enhancing health.

As the results of many researches it has been shown that stigmata of *Zea mays* contains proteins, vitamins, carbohydrates, Mg^{2+}, Na^{+}, Ca^{2+}, and K^{+} salts, volatile oils, and steroids such as sitosterol and stigmasterol, alkaloids, saponins, tannins, and flavonoids [1–3]. The phenolic compounds present in corn silk are anthocyanins, *p*-coumaric acid, vanillic acid, protocatechuic acid, derivatives of hesperidin and quercetin, and bound hydroxycinnamic acid forms composed of *p*-coumaric and ferulic acid [4].

Corn silk virtue has been reported in many examinations. Therefore, in recent years, considerable attention has been directed toward the identification of plants with antioxidant ability that may be used for human consumption. Diuretic, as well as antilithiatic, uricosuric, and antiseptic, properties are traditionally attributed to corn silk, which has been used in many parts of the world for the treatment of edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis [1–3,5,6]. In China, corn silk has been used as an oral anti-diabetic for centuries. In France, it has traditionally been used to stimulate weight loss [7]. There are some literature reviews on yellow corn silk revealing that it contains high concentrations of phenolic compounds and flavonoids. Several reports have been published on biological effects of corn silk such as antiproliferative effects on human cancer cell lines, lipopolysaccharide-induced cell adhesion, antibiotic and antifungal, antioxidant effects, and anti-diabetic activity [8–11].

Recent research shows that corn silk promotes bile flow, has neuroprotective effects against oxidative stress and anti-fatigue activity in animals by inhibiting the production of blood lactic acid, as well as antitumor effect [12–15].

Potential healthcare applications of corn silk are very much related to its chemical composition and mechanism of action of its bioactive constituents such as phenolics and terpenoids, as well as polysaccharides and glycoproteins. Known for their antioxidant and free radical scavenging capacity, flavonoids are major active phenolic compounds and ingredient in corn silk that possess various pharmacological activities [16].

Health benefits of corn silk have been reported in many investigations. Corn silk extract could promote insulin production by animals, support the recovery of the injured cells of the kidney, pancreas and control blood sugar level in rats [17]. Corn silk, a diuretic, can increase the flow of urine from the body and may reduce the risk of new stones developing. It was indicated that corn silk might reduce or even prevent renal damage by defending kidney against oxidative stress [18].

Silk of corn, rich in polyphenol compounds can be used as dietary fiber and as a food additive for the prevention of several diseases [19].

Therefore, the aims of this study were to study the phytochemical screening, determinate the total polyphenol and total flavonoid content of stigmata of *Zea mays* extracts (aqueous/hydro-ethanolic). To the best of our knowledge, no scientific reports on the biological activities of Moroccan corn silk were so far available (which is considered till now in our country as a waste, regardless its medicinal and economic importance). Regarding their antioxidant, anti-inflammatory, antinociceptive and the toxicity for different extracts.

Material and methods

Plant materials

The plant material used for this investigation was fully developed, mature corn of *Zea mays* were collected in July 2015. The corn was dehusked and stigmata were detached from the fruit. Plant material was dried in a shaded and well-ventilated place. The dried stigmata were grounded into a powder form, then kept refrigerated until used.

Plant extraction

Decoction

The extraction method was performed by taking 20 g of dried and pulverized corn silk from *Zea mays* with 200 ml of distilled water. The mixture was heated (reflux system) for

30 min. The mixture was filtered using Whatman filter paper and concentrated under reduced pressure.

Soxhlet extraction

In a Soxhlet system, 25 g of dried plant powder was put in a cellulose cartridge and extracted with 250 ml of ethanol–water (70/30). The extraction process continues until the solvent in siphon tube of an extractor become colorless. After that the extract was filtered and been concentrated under reduced pressure. The extracts were stored at 4 °C for subsequent analyses.

Qualitative phytochemical analysis

Phytochemical screening of dry extracts was achieved through simple methods as described in [20–23]. These tests reveal the presence of a number of chemical groups of the aqueous and hydro-ethanolic extracts.

Quantitative phytochemical analysis

Determination of the total phenolic content

To determine the total phenolic content, we used the method of Folin–Ciocalteu [24], we picked up 20 µl from each extract and mixed with 1.16 ml of distilled water, 100 µl of Folin–Ciocalteu reagent, followed by addition of 300 µl of Na_2CO_3 solution (20%) after 1 min and before 8 min. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. A calibration curve was plotted for different concentrations of gallic acid. The data are presented as the average of triplicate analyses.

Determination of flavonoid contents

The total flavonoid content was determined using the colorimetric aluminum chloride method as adapted by [25]. In short, sample solution (1 ml) was mixed with the same volume of aluminum trichloride (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 ml) to methanol (1 ml) without $AlCl_3$.

The sample and blank absorbances were assayed at 420 nm after 10 min of incubation at room temperature. The absorbance of the blank was subtracted from that of the sample. The total flavonoid content was expressed in milligram equivalents of quercetin per milligram extract (QE µg/mg of extract). The data are presented as the average of triplicate analyses.

Evaluation of the antioxidant activity

Free radical scavenging activity (DPPH)

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-colored methanol solution of DPPH. The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by [26]. Different concentrations of the extracts (0.1 ml) were added to 3 ml of a 0.001 M DPPH in methanol. Absorbance at 517 nm was determined after 30 min.

The antiradical capacity of the extracts studied was calculated using the following formula:

DPPH scavenging effect (%) = $[(A_0 - A_1)]/A_0 \times 100$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

The antiradical activity was expressed as IC_{50}, which is the antiradical concentration required to cause 50% of inhibition. A lower IC_{50} value corresponds to a higher antioxidant capacity of the extract.

The IC_{50} was calculated by plotting inhibition percentages against concentrations of the sample. The experiment was repeated three times and the results were expressed as mean ± SD.

Ferric-reducing antioxidant power (FRAP)

The reducing power of a product is associated with its antioxidant power. This test is realized according to the procedure of Oyaizu [27]. Based on the reduction of Fe^{3+} present in the complex $K_3Fe(CN)_6$. The absorbance is determined at 700 nm. An increased absorbance indicates ferric reducing power capability of sample. In a test tube, 0.5 ml of extract solutions, standard or blank were mixed with 2.5 ml of sodium phosphate buffer (0.2 M; pH = 6.6) and 2.5 ml of a solution of ferricyanure of potassium (1%). The mixture is brooded during 20 min in a thermostated bath at 50 °C. After cooling, 2.5 ml of trichloroacetic acid (10%) are added to the mixture. Finally, 2.5 ml of $FeCl_3$ (1%) was added to this solution. Absorbance of this mixture was measured at 700 nm using a UV spectrophotometer.

Evaluation of the total antioxidant capacity by phosphomolybdenum method

Total antioxidant capacity (TAC) of the extracts was evaluated by phosphomolybdenum method Prieto [28]. This technique is based on the reduction of molybdenum Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. A volume of 0.3 ml of the extract was mixed with 3 ml of the reagent solution

(0.6 M sulfuric acid, 28 mM sodium and 4 mM of ammonium molybate). The tubes were incubated at 95 °C for 90 min. After cooling, the absorbance of the solutions is measured at 695 nm against the blank which contains 3 ml of the reagent solution and 0.3 ml of methanol and which was incubated under the same conditions as the sample.

Total antioxidant capacity is expressed in milligrams of ascorbic acid per gram of dry matter (mg EAA/g MS). The experiments are repeated three times.

Animals

Wistar female and male rats were acclimatized in cages, kept in well-ventilated environment and had free access to water and food *ad libitum*, and were housed in a quiet room a "12-h light: 12-h dark" cycle for 2 weeks before experimentations. The negative control group received saline (10 ml/kg, i.p.).

Toxicity

Oral acute toxicity

The rats were divided into three groups of 3 males and 3 females ($N = 6$) weighting 100–150 g. Toxicological tests were evaluated by the preparation of different concentrations of the extract which were administered orally (250, 500, 2,000 mg of hydro-ethanolic extract/kg b.w.). The general behavior of rats and signs of toxicity were observed continuously for 1 h after the oral treatment, and then intermittently for 4 h and thereafter over a period of 24 h [29].

The rats were further observed once a day up to 14 days for following treatment for behavioral changes and signs of toxicity and/or death, and the latency of death. The LD_{50} value was determined according to the method of [30].

Sub-acute oral toxicity

For the sub-chronic study, 6 adults Wistar rats (150–200 g) were randomly placed in a cage under the same conditions as mentioned above. They received a daily gavage at doses of 250–500 and 2,000 mg/kg of hydro-ethanolic extract of stigmata of *Zea mays* for 28 days. The animals were observed for 2 h for any behavioral changes, neurological and autonomic profiles or cases of death after 24 h. The general behavior of the animal, the weight, the morphological appearance of organs (liver, spleen, and kidneys), and the relative organ weights (ROW) in comparison with the control group, calculated by the following formula:

$$ROW = (\text{organ weight}/\text{body weight}) \times 1000)$$

Anti-inflammatory study

The method used was similar to that previously reported by [31]. Rats were divided into four groups of five animals each. Group I served as the control group receiving normal saline. Groups II and III were given at doses of 250 and 500 mg/kg by hydro-ethanolic extract. Animals in group IV were treated by indomethacin 10 mg/kg. One hour later after application 0.1 ml of 0.5% carrageenan (in normal saline) injected subcutaneously into the plantar aspect of the right hind paw of each rat.

Measurements of paw size were performed immediately 3, 4, 5, and 6 h after injection of carrageenan. The edema degree in the hind paw for each rat, and percentage inhibition for each group, were calculated according to the following formulae:

$$\text{Oedema degree (E)} = V_t - V_0$$

$$\% \text{ inhibition} = (Ec - Et)/Ec$$

V_t: is the paw size after carrageenan injection at t hours; V_0: is the paw size before carrageenan injection; Ec: is the mean edema degree of the control group; Et: is the mean edema degree of the treated group.

Antinociceptive activity

Formalin test

The method used in the present study was similar to that described previously by [32]. It consists briefly of injecting subcutaneously 20 µl of 20% formalin into the right posterior paw of mice placed in a transparent enclosure. Throughout 5 min prior to this procedure; each mouse is allowed to adapt the testing box and left freely moving and exploring (habituation). The formalin induced licking of the paw was considered as indicative of the nociceptive behavior. Using a chronometer, the total time spent in licking and biting the injected paw is recorded, quantifying the nociceptive behavior. However, as the formalin test in rodent consists of two successive phases [33]. The initial nociceptive response normally peaked 5 min after formalin injection (early phase) and 15–30 min after formalin injection (late phase), representing the tonic and inflammatory pain responses, respectively.

The animals were pre-treated intraperitoneally with the extract (500 mg/kg), or with tramadol (10 mg/kg), 30 min beforehand.

Statistical analysis

Data was expressed as means ± standard of triplicate analysis ($N = 3$). The statistical analyses were performed by student's *t*-test. The values of P lower than 0.05 were considered statistically significant.

Results and discussion

Phytochemical screening

As shown in table 1, the phytochemical tests carried out on extracts of stigmata of *Zea mays* indicated the presence of phytoconstituents such as flavonoids, leucoanthocyans, heterosid sterodic, coumarins, alkaloids, cardiac glycosides, anthocyanins and tannins. They were present in both hydro-ethanolic and aqueous extracts. Whereas sterols, terpenoids, oses and holosides were only detected in hydro-ethanolic extract. However, mucilage was noticed only in aqueous extract. Nevertheless, saponosids was not observed in both extracts.

Even so, corn silk found in Malaysian has been reported to contain phenols, flavonoids, tannins, alkaloids, saponins and cardiac glycosides [34]. However, Thoudam et al. [35] has hound flavonoids, alkaloids, phenols, steroids, glycosides, carbohydrates, terpenoids and tannins. In addition, saponin, phlobatannins, tannins, polyphenols and steroids were detected in Malaysian corn silk [36].

Yield and determination of total phenolic and flavonoids content

For both hydro-ethanolic and aqueous extracts, the mass yield obtained were 20.7% and 9.55%, respectively (w/w). The results show that the yield of the hydro-alcoholic extract was higher than the aqueous extract. The masse obtained by Soxhlet extraction was the higher because of the heating and long extraction time.

Concerning the total phenolic (TPC) and flavonoids content it was found that hydro-ethanolic extract contains more amounts of these phytochemicals (Table 2). The TPC was determined through a linear gallic acid standard curve ($Y = 1.575X - 0.0229$; $R^2 = 0.959$). The results expressed in terms of mg GA/g of extract. We found that the hydro-ethanolic extract has better content (75.20 ± 1.83 mg AG/g extract) than the aqueous extract (41.54 ± 7.90 mg AG/g extract).

The value TPC of the corn silk extract in our study was estimated to be higher than the corn silk extract reported by other researches [37–38]. Despite that our results are lower than those found by [38].

The amount of polyphenolic compounds in different extracts dependent on its origin [39–40], and was influenced by a various parameter. Extraction process involved separation of active fractions from plant tissue by using selective solvents and extraction methods [41].

The total flavonoid content was determined in comparison with quercetin. The hydro-ethanolic extract has higher flavonoid content than that of the aqueous extract ($Y = 0.0489X - 0.0416$; $R^2 = 0.977$).

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of the actions of flavonoids are through scavenging or chelating processes. Phenolic compounds are a class of antioxidant agents acting as free radical terminators [42–44]. Still, there are primary factors influencing the variability of phytochemicals in plants comprising genotype, size and maturity, soil conditions, fertilization, irrigation, pesticide utilization, disease and pests, location and climate, and season. Thus, these factors can be applied to improve and enhance phytochemical content in plants [45].

Evaluation of the antioxidant activity

Free radical scavenging activity (DPPH)

The hydrogen donating or radical scavenging ability was assessed by using DPPH assay. As a kind of stable free

Table 1 Phytochemical screening of hydro-ethanolic and aqueous extracts of stigmata of *Zea mays*

Chemical constituent	Hydro-ethanolic extract	Aqueous extract
Tannins	+	+
Catechic tannins	+	+
Gallic tannins	−	−
Flavonoids	+	+
Leucoanthocyans	+	+
Saponosids	−	−
Sterols and terpens	+	−
Heterosid sterodic	+	−
Triterpens heterosids	−	−
Coumarins	+	+
Alkaloids	+	+
Cardiac glycosides	+	+
Oses and holosides	+	−
Mucilage	−	+
Anthocyanins	+	+

Table 2 The mass yield, total phenolic content (TPC) and total flavonoids content (TFC) of *Zea mays* extracts

Extract	Mass yield (%)	TPC (mg AG/g extract)	TFC (mg Q/g extract)
Hydro-alcoholic	20.7	75.20 ± 1.83	25.95 ± 0.36
aqueous	9.55	41.54 ± 7.90	6.99 ± 1.268

radical, DPPH can accept an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical scavenging activity. The antioxidants can react with DPPH, a deep-violet colored stable free radical, converting it into a yellow colored α, α-diphenyl-β-picrylhydrazine.

The discoloration of the reaction mixture can be quantified by measuring the absorbance at 517 nm, which indicates the radical-scavenging ability of the antioxidant [26].

The anti-free radical capacity of the hydro-alcoholic and aqueous extracts was tested by the DPPH method using BHT as a reference standard. The concentration varies between 0.01 and 6 mg/ml. Inhibition zero was considered for the solution that contained only DPPH without plant extract. The result showed strong antioxidant activity for the hydro-ethanolic extract compared to the aqueous extract, the detailed results are presented in table 3.

Hydro-alcoholic extract exhibited a good reducing power and was also a good bioactive source of natural antioxidants. Our results are similar to those found by other researchers, the hydro-ethanolic extract exhibited higher level of scavenging activity compared to the aqueous extract [8,38].

The DPPH scavenging activity showed a similar trend with the content of total phenolic compounds and total flavonoids, indicating that the phenolic compounds, particularly flavonoids that present in *Zea mays* are the major constituents which can scavenge the DPPH radical, due to the presence of the hydroxyl groups in their structure and their electron donating ability. These results were consistent with the findings of many research groups, who reported such correlations between total phenolic content and free-radical scavenging activity [46–47].

Reducing power by FRAP method

The potassium ferricyanide reduction method was used as an indicator of electron donating activity to evaluate the reducing power of plant polyphenols, which is a widely used method in measuring antioxidant activity of phenolic compounds.

In this assay, the antioxidants presented in the test solution can reduce the Fe^{3+}/ferricyanide complex to the ferrous form by donating an electron. The color of the tests solution then changes from yellow to different shades of green and blue, which depends on the reducing power of the extracts of corn silk [27]. Increasing absorbance of the reaction mixture at 700 nm indicates an increase in the reducing power.

Table 4 shows an antioxidant activity for the hydro-alcoholic extract with an $IC_{50} = 2.46$ mg/ml, and an $IC_{50} = 3.25$ mg/ml for the aqueous extract comparable to BHT the standard which has an $IC_{50} = 1.68$ mg/ml. The hydro-alcoholic extract showed stronger the reduction of power compared to the aqueous extract, the results are presented in table 4.

Significant correlation has been found between the reducing power activity and the TPC ($R = 0.9792$) and TFC ($R = 0.9838$) of corn silk fractions, indicating that the reducing power is highly related to the amount of phenolic compounds especially flavonoids that present in the extracts, which can serve as electron donor to terminate the radical chain reaction [8].

Evaluation of the total antioxidant capacity by phosphomolybdate method

The total antioxidant activity of aqueous and hydro-ethanolic extracts is expressed in equivalents of BHT (Table 5). The test is based on the reduction of Mo (VI) to Mo (V) by the extract and the subsequent formation of a phosphate of a complex green/Mo (V) complex at an acidic pH. The method is quantitative phosphomolybdate since the antioxidant activity is expressed in the number of equivalents of BHT [21]. The hydro-ethanolic extract has a capacity greater than that of the aqueous extract.

Our results showed higher values than that found by Liu et al. [8] which obtained a total antioxidant activity of 60.74 ± 4.21 µg/ml.

Table 3 Inhibition activity of BHT and different extracts of stigmata of *Zea mays* by DPPH method

Extract	IC_{50} (mg/ml)
Hydro-ethanolic	2.34
aqueous	5.83
BHT	0.17

Table 4 Inhibition activity of BHT and different extracts of stigmata of *Zea mays* by FRAP method

Extract	IC_{50} (mg/ml)
Hydro-ethanolic	2.46
Aqueous	3.25
BHT	1.68

Table 5 Total antioxidant activity of extracts of stigmata of *Zea mays* ($N = 3$)

Extract	EC_{50} (mg BHT/g of extract)
Hydro-ethanolic	52.37 ± 5.24
Aqueous	22.67 ± 2.27

Toxicity

Oral acute toxicity

During the experiment, no toxic symptoms or morbidity was observed in any animals during the 14 consecutive days of the treatment. Therefore, the acute minimum lethal dose (LD_{50}) of the hydro-ethanolic extract of corns silk for Wistar rats is higher than 2,000 mg/kg b.w. The same results have been found by [3] with concentration that varied from (0.5–4 g/kg b.w.), so we can deduct that the LD_{50} is 4 g/kg b.w.

Sub-acute oral toxicity

During the experiment, the rats did not show any observable signs of toxicity or morbidity. Furthermore, no mortality was recorded. There was significant difference in the ROW of liver compared to the control, and also significant for kidney in the dose of 2,000 mg/kg b.w. and in the spleen for the doses 250–500 mg/kg b.w. For the rest of the organs there was no significant difference in the ROW of the treated groups of rats when compared to the control group (Table 6).

Anti-inflammatory study

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 h. The increase of edema was greater in control group (saline) than the treated groups (extracts and indomethacin). At doses of 250 and 500 mg/kg b.w. extracts produced a maximum and higher inhibition of carrageenan induced inflammation of 66.67% and 86.67%, respectively, compared to indomethacin which produced an inhibition of 60.48% (Fig. 1).

Carrageen an induced rat hind paw edema has been widely used for the discovery and evaluation of anti-inflammatory drugs because the relative potency obtained from most drugs tends to reflect clinical experience [31]. This suitable test also has frequently been used to access to the antiedematous effect of natural products [48]. The local injection of carrageenan-induced inflammatory process in the rat involves three phases by several mediators released in ordinate

Table 6 Relative organ weights of rats after treatment with hydro-ethanolic extract of *Zea mays*

Extracts	Dose (mg/kg)	Body weight (g)	Relative organ weight (ROW)		
			Liver	Kidney	Spleen
Hydro-ethanolic	250	118.6 ± 1.073	39.29 ± 1.61**	7.40 ± 0.45*	2.37 ± 0.26**
	500	156 ± 4.32	41.39 ± 5.37**	6.92 ± 0.41*	1.98 ± 0.16**
	2,000	147.33 ± 6.13	47.14 ± 7.65**	7.45 ± 0.94**	3.26 ± 0.33*
Control	0	209 ± 8.37	44.69 ± 5.53	8.61 ± 0.83	2.01 ± 0.34

*$p < 0.01$; **$p < 0.001$

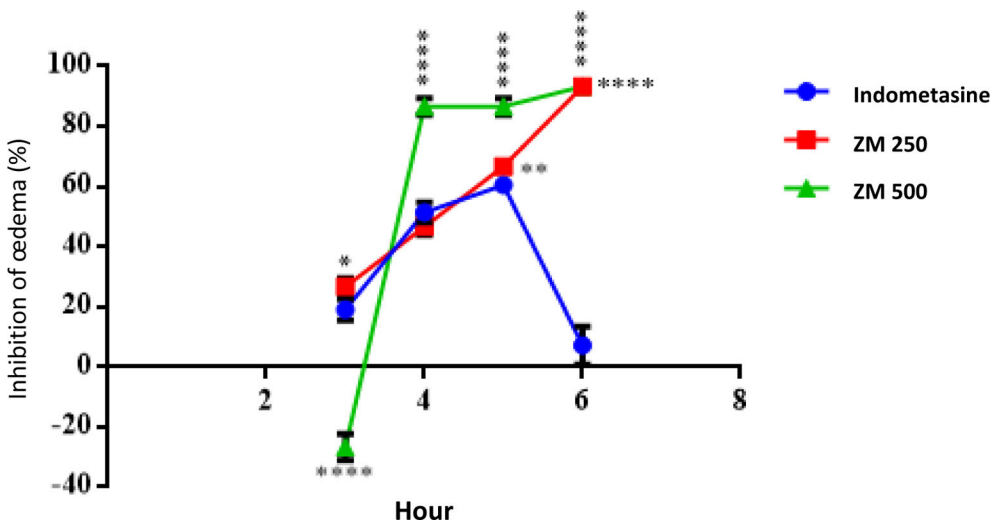

Fig. 1 Anti-inflammatory effect of extract of stigmata of *Zea mays* compared to indomethacin $N = 5$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$

sequence [49]. An initial phase, during the first 1.5 h, is caused by the release of histamine and serotonin, a second phase is mediated by bradykinin from 1.5 to 2.5 h and finally, and a third phase, the mediator of which is suspected to be prostaglandins, occurs from 2.5 to 6 h after carrageenan injection. This third phase seems to be the most interesting phase. Thus, the maximal vascular response, as determined with leukocyte migration to the inflamed area, also reaches its maximum level in this third phase [50]. It is well established that prostaglandins, by virtue of their activity as modulators of inflammatory responses, have a major role in the inflammatory mechanism.

The anti-inflammatory activity of extract is due to their richness in total polyphenols, flavonoids, tannins and mucilage. The anti-inflammatory of flavonoids and mucilage has been proven by several studies [51]. The significant anti-inflammatory effect of our extract observed after 5 h and 6 h is prolonged and its bioavailability is higher compared to indomethacin.

Antinociceptive activity

Formalin test

In the formalin test, the most anti-inflammatory effective dose (500 mg/kg b.w.) was chosen to investigate the antinociceptive activity of the hydro-ethanolic extract.

The tolerance time of the rats treated with the extract was significantly higher ($P < 0.001$) compared to the control group. We noted a slight difference between Tramadol and hydro-ethanolic extract (Fig. 2).

The formalin test involves two phases: a neurogenic phase with release of substance P, and an inflammatory phase with release of serotonin, histamine, bradykinin and prostaglandins [52]. This is of interest considering that both phases are sensitive to centrally acting drugs, such as opioids [53]. However, the second phase is also sensitive to NSAIDs (non-steroidal anti-inflammatory drugs) and corticosteroids [33]. In addition, it is generally agreed that *N*-methyl-D-aspartate (NMDA) receptors contribute to the persistent chemical stimulus during the late-phase central sensitization of dorsal horn neurons [54]. Most drugs capable of reducing the excitability of spinal cord neurons, including opioids, NSAIDs and glutamate antagonists, can also reduce or even abolish wind-up [55].

It has been demonstrated that the nociception produced by formalin (first phase) is quite resistant to the great majority of non-steroidal anti-inflammatory drugs, while it is sensitive to dipyrone, opioid drugs such as morphine and drugs that antagonize substance P or glutamate receptors [56–59]. It has been reported that flavonoids isolated from several medicinal plants are responsible of the analgesic and anti-inflammatory activities [60–61]. Indeed, quercetin was described to involve α-2 adrenergic [62] and D2 dopaminergic [63] receptors in mediating the antinociceptive activity in thermal and chemo-nociceptive assays. It has been shown also that catechins have analgesic properties [64].

We notice a significant antinociceptive activity of the hydro-ethanolic extract of stigmata of *Zea mays* at a dose

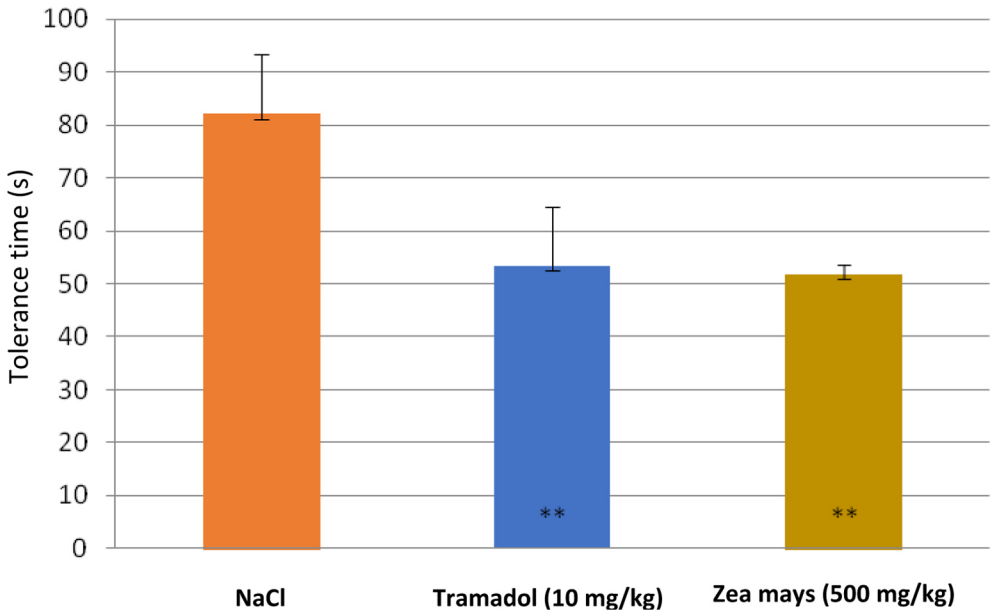

Fig. 2 Antinociceptive effect of hydro-ethanolic extract compared to tramadol and control group $N = 5$; $^{**}p < 0.001$

of 500 mg/kg compared to the medicament which is the tramadol with a dose of 10 mg/kg.

Hydro-ethanolic extract was effective as an anti-inflammatory and analgesic compound in various pain models, probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms (opioid system). Further studies currently in progress will enable us to understand the precise action mechanisms.

Conclusion

This study shows that the hydro-ethanolic extract of stigmata of *Zea mays* has an important antioxidant power, also possesses antinociceptive and anti-inflammatory activities with effective dose of 500 mg/kg, the evidence of possible involvement of peripheral and central effects in its actions. These findings support the use of *Zea mays* in traditional medicine for the treatment of some pathology like edema as well as for cystitis, gout, kidney stones, nephritis, and prostatitis. Also, confirmed the presence of biologically active principles whose activities need further investigation. The plant extract at doses tested also seems to present varied/or different central and peripheral mechanism of action, which may worth further elucidation and evaluation.

Conflicts of interests: the authors have no conflicts of interests to declare.

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