Phytochemical screening and in vivo evaluation of anti-inflammatory potential of methanolic extract of *Gleditsia triacanthos*

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Abstract

Plants contain many phytochemical constituents that show various biological activities. The primary objective of this study is to determine the bioactive compounds of the extract of the pods (fruits) of *Gleditsia triacanthos* (honey locust); which is widely used in medicine. In the second step, the anti-inflammatory activity of these compounds was determined *in vivo* on Wistar rats. Phytochemical screening tests showed that the extracts of *G. triacanthos* are rich in a few groups mainly: total tannins, flavonoids, coumarins and saponosids. The results of the quantitative analysis showed that the methanolic extract is rich in polyphenolic compounds with significant contents of the total polyphenols of 14.73 ± 2.51 mg GAE / g, flavonoids of 9.65 ± 0.52 mg QE / g and condensed tannins of 6.95 ± 2.64 mg CE / g. The results of the *in vivo* evaluation of anti-inflammatory activity showed that methanolic extract resulted in a reduction in inflammatory reaction in the dextran induction model. It is manifested by an inhibition percentage of inflammation ranging from 6.56 to 10.76%. The present study showed that extract of *G. triacanthos* has an anti-inflammatory activity justifying their use in traditional medicine. It is therefore of considerable therapeutic interest as an alternative compound for the prevention of inflammation.

Keywords: *Gleditsia triacanthos*, Extraction, Polyphenols, Anti-inflammatory activity.

Received: 22/ 10/ 2020

Revised: 01/ 12/ 2020

Accepted: 24/ 12/ 2020

Introduction

Many aromatic and medicinal plants and spice plants, cultivated or spontaneous, have biological properties which allow their applications in various fields, including medicine, pharmacology, cosmetology, and agriculture (Valnet, 2001). The evaluation of the biological effects of these plants has shown that some have very interesting therapeutic uses. Several plant extracts have been studied for the treatment of diseases generated by oxidative stress and inflammatory mechanisms (Hannebelle, et al., 2007). Indeed, the scientific world declared a new concept, that of oxidative stress, a situation thus causing panoply of diseases, such as inflammation. Previous studies showed that plants containing chemical compounds called secondary metabolites are divided into several classes, among the most studied, polyphenols, are recognized as very good anti-inflammatory agents (Bouayed, et al., 2007). Medicinal plants and their secondary metabolites with antinociceptive properties have been used as the most highlighted therapy for the inflammatory mechanisms (Almeida, et al., 2012). Recent results suggested that polyphenols can act on gene expression as well as on many signaling pathways involved in pathological processes such as inflammation (Belaid and Bellil, 2017). For this reason, it is necessary to discover natural anti-inflammatory drugs which can be substituted for anti-inflammatory of chemical synthesis that accompanied by undesirable side effects.

Gleditsia triacanthos L. (Honey locusts) is a deciduous tree belonging to the family of Fabaceae. G. triacanthos pods have been used as a folk remedy for measles and dyspepsia among the Cherokee tribe (Lim, et al., 2005). The Honey locust was used for food by the native American people (Mohammed, et al., 2014). In the perspective of researching and developing anti-inflammatory phytomedicines from medicinal plants, our work was focused to evaluate the anti-inflammatory activity of the methanolic extract of the fruit of Gleditsia triacanthos using induced model of 1% dextran.

Materials and Methods

Plant Material

Fruit (pods) of the G. triacanthos plant was harvested in the East region of Tighennif, Mascara, Algeria in January 2019 (Latitude: 35.4167, Longitude: 0.333 with 35° 25′ 00″ north, 0° 19′ 59″ east). The plant was identified by botanists of the Department of Biology of the University of Mustapha Stambouli, Mascara.

Experimental Animals

The model chosen is the albino wistar rat, with an average body weight of 200 ± 15 g, which was obtained from pet of the University of Mustapha Stambouli, Mascara. The mice are divided into batches and are kept at a temperature of 24 ± 1 °C with a natural photoperiodic cycle (12h of light and 12h of dark). They are fed with food pellets and water ad-libitum with care and treatment conditions in accordance with the guidelines of the Organization for Economic Cooperation and Development (OECD, 2004).

Preparation of Methanolic Extract

The protocol adopted for the extraction is that of Upson, et al. (2000). The crude extract was obtained by maceration of 20 g of the vegetable powder of Gleditsia triacanthos fruit with 200 ml of methanol 80% for 24 h under magnetic stirring. Then, the solution was filtered on filter paper. Then, the filtrate was concentrated in a rotary evaporator followed by drying in the oven and the extract was collected in sterile and hermetically bottles. The extraction yield was then calculated.

Phytochemical Screening

Qualitative Analysis

This was a qualitative study aimed to finding the main chemical groups (alkaloids, flavonoids, coumarin and tannins). Characterization tests were based on precipitation and complexation reactions with the formation of insoluble and colored complexes. The observed coloration was caused using of an appropriate reagent and was generally due to the reaction between molecules (Harbone, 1998; Rai and Carpinella, 2006).

Quantitative Analysis

Total Phenolic Content (TPC)

The determination of total polyphenols was carried out according to the Folin-Ciocalteu (FC) method (Benhamou, et al., 2008). 1.25 ml of Folin-Ciocalteu reagent was added to 1 ml of sodium carbonate (Na2CO3) 2% and 0.25 ml of Methanolic extract, incubated

at room temperature 90 min. The absorbance is measured at 760 nm. The results were expressed in mg Gallic Acid Equivalent / g with reference to the gallic acid calibration curve. The Calibration range was prepared with dilution series (50/50) of standard gallic acid solution (200 mg / l).

Total Flavonoids (TF)

The determination of total flavonoids was carried out by the aluminum trichloride ($AlCl_3$) method according to the protocol of Dewanto, et al. (2002). 1 ml of diluted extract (0.01 g / 10 ml of the same extraction solvent) added to 0.3 ml of sodium nitrite ($NaNO_2$). Then, after 5 min, 0.3 ml aluminum trichloride ($AlCl_3$) was added. Then 2 ml of sodium hydroxide (NaOH) was added. The absorbance was measured at 510 nm. The results were expressed in mg Quercetin equivalent / g with reference to the Quercetin calibration curve. The Calibration range was prepared with dilution series (50/50) of standard Quercetin solution (200 mg / l).

Condensed Tannins (CT)

The content of condensed tannins was determined using the vanillin spectrophotometric method (Julkunen-Titto, 1985). A total volume of 0.5 ml of polyphenolic or standard extract (catechin) added to the mixture of 3 ml to vanillin 4%, 1.5 ml of hydrochloric acid and then homogenized. The mixture was left for 15 min at room temperature. Then, the absorbance of each extract was calculated at 500 nm. The total content of condensed tannins was expressed in mg Catechin Equivalent / g using the equation obtained from the calibration curve.

Acute Toxicity

A total of 60 wistar rats (males and females) weighing 200 ± 15 g were used. Acute toxicity was estimated using the method described by Tahraoui, et al. (2010), which consist of dividing the mice into 12 groups (6 for males / 6 for females) of 5 rats each. The rats were previously deprived of food for 18 h. They just had access to water. Then, each animal was identified and weighed. The concentrations of the Methanolic extract were: 250, 500, 1000, 1500 and 2000 mg / kg of body weight. The different doses were administered orally to the five groups with 10 ml / kg. A group representing the control batch treated with saline solution. After the administration of the extracts, the rats were observed individually every 30 minutes for 6 h, the first day and every day for 15 days (Adjoungoua, et al. 2008). The number of dead rats as well as the behavior and symptomatic disorders are recorded.

In vivo anti-inflammatory activity

The evaluation of the anti-inflammatory effect was based on the method of induction of edema by the injection of dextran in the paw (Fereidoni, et al., 2000). To achieve this type of inflammation, 09 rats with body weight of 200 ± 15 g were used. They were divided into three groups: the first group used for the control treated with saline (0.9% NaCl). The second treated with Methanolic extract of *Gleditsia triacanthos* fruit (250 µl / kg) and the last group was used in the standard treated with Ibuprofen® at a dose of 10 mg / kg (250 µl / kg). The different treatments were administered orally one hour before the dextran injection. After one hour of the treatments, the thickness of the paw was measured before and 1, 2 and 3 h after the injection of dextran using a caliper. The volume of the edema was deter-

mined and expressed as a percentage of swelling, based on the initial volume of the paw of each rat. Then, the inhibition percentage was calculated according to the following formula:

$$\text{Inhibition percentage (\%)} = DC\text{-}DT \times 100 / DC$$

DC: Diameter of the control, DT: Treated paw diameter

Results

Extraction Yield

The extraction of the polyphenols from the fruits of *G. triacanthos* with 80% methanol gave a yield of 32.82%. These results testified to the richness of the pods of *G. triacanthos* in phenolic compounds. In agreement with these results, several authors showed that aqueous methanol remains the most suitable solvent for extracting phenolic compounds from a plant (Sun, et al., 2007).

Phytochemical Screening

Qualitative Analysis

The results of the qualitative study of the methanolic extract of *G. triacanthos* were expressed in Table 1. They revealed that all the chemical groups are strongly characterized using methanolic extracts. There is also a strong presence of total tannins and flavonoids. Coumarins, saponosids, and anthocyanins are poorly characterized while Irridoids were undetectable.

Table 1: *Phytochemical tests of Methanolic extract of G. triacanthos*

	Metabolite	Extract
01	Anthocyans	+
02	Total Tannins	++++
03	Irridoids	-
04	Flavonoïds	+++
05	Coumarins	++
06	Saponosids	++

Quantitative Analysis

Quantitative analysis was determined from the linear regression equation of the calibration curve (Table 2). The results showed a total polyphenol content equal to 14.73 ± 2.51 mg GAE/g.

The flavonoid content was relatively higher in the extract (9.65 ± 0.52 mg QE / g). These results were close to those reported by Saidi, et al. (2018) with a value of 10.72 ± 0.56 CE / g in the methanolic extract of *G. triacanthos*, while the determination of the condensed tannins showed that methanolic extract gave a value of 6.95 ± 2.64 (mg CE/g).

Table 2: *Quantitative analysis of Methanolic extract of G. triacanthos*

	TPC (mg GAE/g)	TF (mg QE/g)	CT (mg CE/g)
Methanolic extract of *G. triacanthos*	14.73 ± 2.51	9.65 ± 0.52	6.95 ± 2.64

Acute Toxicity

After administration of different doses of the methanolic extract of *G. triacanthos* (250, 500, 1000, 1500 and 2000 mg / kg) no delayed effect was reported in rats compared to the control group. The experiment did not show any serious clinical symptoms of suffering despite some usual signs observed such as hypoactivity. They were reversible and appeared in rats for a short time and then returned to activity.

In vivo Anti-inflammatory Activity

The anti-inflammatory activity was determined by monitoring the evolution of the paw thickness of the rats of each group. The injection of dextran after 1 h of the administration of saline and the different treatments (methanolic extract of *G. triacanthos* and Ibuprofen) leads to an increase in the paw thickness of the rats from first hour of the experiment: for the control (h1 = 7.99 mm), the extract (h1 = 7.21 mm) and Ibuprofen (h1 = 7.80 mm) (Figure 1). After 6 h of treatment with methanolic extract of *G. triacanthos* and Ibuprofen, the results showed a restoration of the paw thickness with a value of 4.4 ± 0.12 and 4.37 ± 0.04 mm with methanolic extract of *G. triacanthos* and Ibuprofen respectively (Figure 1). All treatments produced reductions in inflammation ranging from 6.56 to 10.76% for methanolic extract of *G. triacanthos* and 5.2 to 8.75% for Ibuprofen (Figure 2). Treatment with methanolic extract of *G. triacanthos* (250 mg / kg) has a very high inhibition percentage of edema at the second hour (15.42%) compared to the first hour (9.79%). Administration of Ibuprofen showed interesting inhibition of paw edema at the second hour (12.31%) compared to the sixth hour (2.67%) (Figure 1 and 2).

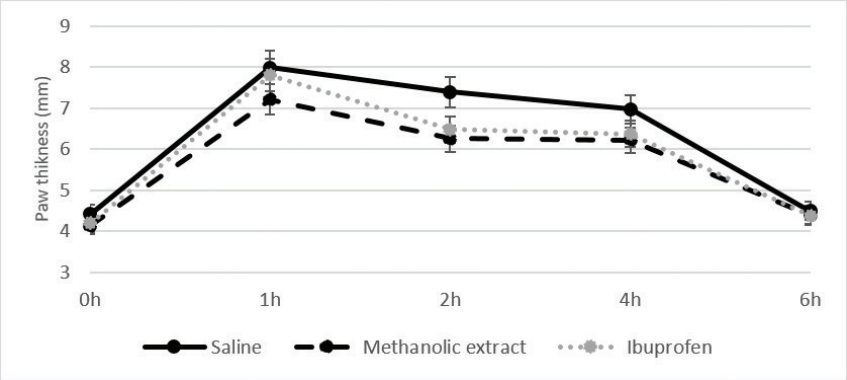

Figure 1: *Evolution of the paw thickness of treated rats*

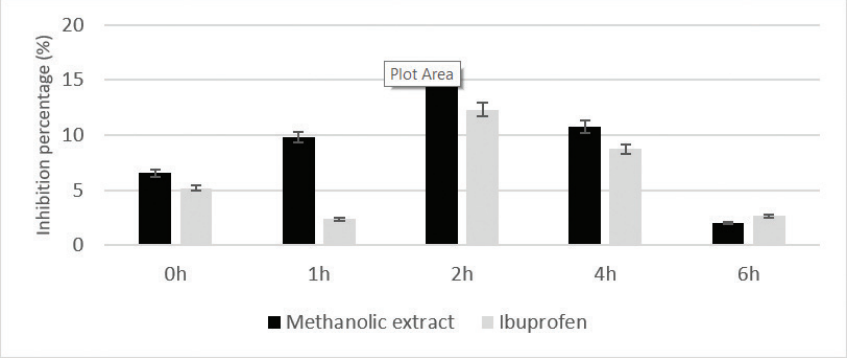

Figure 2: *Inhibition percentage of edema in treated rats*

Discussion

Many studies confirmed that the use of mixed solvents (hydro-alcoholic) leads to a strong enrichment of the extracts in polyphenols (Mohammedi and Atik, 2011). Variations in the extraction yield can be related to several factors such as the origin, the geographical area, the harvest period of the plant, the extraction method and the extraction conditions under which it was carried out (Ebrahimi, et al., 2008; El-Hashash, et al., 2010). The study of Leibovici, et al. (1986) confirmed the presence of bioactive compounds mainly the heterosides of the C-glycosyl type particularly present in Gleditsia genus. Mohammed et al. (2013), isolated six glycosylated flavonoids and two aglycones from the leaves of *G. Triacanthos*. According to Kamalak, et al. (2012) fruit of *G. triacanthos* contained significant levels of condensed tannins. Previous studies have reported the presence of saponosids in different fruits of the Gleditsia. Indeed, according to Zhang, et al. (2016), 32 compounds out of 67 identified would be saponosids. Compared to the study by Saidi, et al. (2018), the determination of total polyphenols revealed contents of 23.83 ± 3.17 and 48.73 ± 0.86 mg GAE / g for the aqueous and methanolic extract of *G. triacanthos* respectively. They appeared superior to those determined in the current study. The contents recorded by Benhamiche et al. (2016) in the methanolic extract of the pods of *G. triacanthos* were significantly lower (0.16 ± 0.06 mg GAE / g). The results of El-Sayed, et al. (2013) on extracts of *G. triacanthos* leaves, indicated a much higher content (240.32 ± 1.56 mg GAE / g) in the crude methanolic extract. In contrast, the results of El-Sayed et al. (2013) on the methanolic extract of *G. triacanthos* indicated a higher content of flavonoids (23.53 ± 0.18 mg QE/g). Many studies indicated higher levels of condensed tannins in the fruits of *G. triacanthos*. Eisenman, et al. (2013) estimated that the fruits of *G. triacanthos* contained 3.1%, while Kaya et al. (2016) indicated even higher levels of condensed tannins (8.35%) in the fruit of *G. triacanthos*.

The absence of serious clinical signs and of dead rats during the observation period indicated that the extract of *G. triacanthos* is devoid of acute toxicity in rats. These results were confirmed by the work of Tahia, et al. (2013), who recorded no mortality when rats where treated with a dose of 2000 mg / kg. Dalia, et al. (2016), also confirmed these results by the absence of any signs of mortality after the administration of a dose of 2000 mg / kg. Since there are no signs of mortality, it is assumed that the LD50 was greater than 2000 mg / kg. In the current study, dextran can cause edema when injected in the sub-plantar part of the paw. The effect of this reaction was tissue damage which induced the synthesis of histamine, prostaglandins, leukotrienes, PAF (platelet activation factor), cytokines, Nitrogen Monoxide and TNF (tumor necrosis factor) (Clarke, et al. 1996). From 0-2 h, the inflammatory mediator was prostaglandin (in particular PGE2) and thromboxane B2 (Ouédraogo, et al., 2012). The injection of 1% dextran caused a biphasic response. The first early phase of inflammation was mainly mediated by chemical mediators such as histamine and serotonin and was characterized by the stimulation of C fibers and the release of substance P and bradykinin. The second late phase (3-4 h after the injection of dextran) was associated with the activation of leukotrienes, prostaglandins and several cytokines (Lu, et al., 2012). The vascular response reached its maximum level in this phase. The anti-inflammatory activity of the extract of *G. triacanthos* can be mediated by inhibition of the late phase by restricting the production of several cytokines, bradykinins, leukotrienes, and prostaglandins (Hoogstraate, et al., 2003). Previous studies have found that anti-inflammatory activities could be

resolved by a reduction in the production of nitric oxide and pro-inflammatory cytokines with a simultaneous increase the level of anti-inflammatory cytokines. However, the reference drug, Ibuprofen, was more effective in preventing these effects. Acute pain can be effectively relieved by Non-Steroidal Anti-Inflammatory drugs that inhibit cyclooxygenase (COX-1 and COX-2) (Lu, et al., 2012). According to Tahia, et al. (2013), treatment with *G. triacanthos* extract at a dose of 400 mg / kg can present significant edema inhibition of 39.69% 4 h after administration of dextran. Based on the results from the phytochemical analysis, it was noted that the anti-inflammatory potential was attributed to the action of polyphenol fractions (Flavonoids, tannins or saponins). This was confirmed by studies carried out on the fruits of *G triacanthos* which have shown that the anti-inflammatory activity was attributed to extracts of *Gleditsia triacanthos* rich in saponins (Yassin, et al., 2013). It should be noted that Non-Steroidal Anti-Inflammatory drugs mainly suppress the last phase of inflammation (prostaglandin phase) which was correlated with their ability to suppress mononuclear leukocytes. Nonsteroidal anti-inflammatory drugs blocked the synthesis of prostaglandins by inhibiting cyclooxygenase and arachidonic acid (Katzung, 1992).

The results of Abou zeid, et al. (2018) of Phytochemical screening of *G. triacanthos* extract revealed presence of sterols and triterpenes which contains appropriate percentage of α-tocopherol, isophytol and β amyrin. α-Tocopherol has been shown to have anti-inflammatory effects both in vitro and in vivo to decrease release of proinflammatory cytokines. β-Amyrin is a triterpenoid and was found to be the precursor of boswellic acid and ursolic acid which have strong 5-lipooxygenase inhibitor.

Conclusion

All these results show that the methanolic extract of *G. triacanthos* exerts an important anti-inflammatory effect. *G. triacanthos* could be used as a natural compound alternative to synthetic anti-inflammatory drugs, in the prevention against inflammation. However, further research studies were recommended to isolate and define the bioactive anti-inflammatory principles of *Gleditsia triacanthos*. Clinical studies were encouraged to evaluate these pharmacological activities.

Acknowledgement

The authors would like to express their sincere gratitude to all staff of the department of Biology, University of Mustapha Stambouli, Mascara.

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الفحص الكيميائي النباتي والتقييم في الجسم الحي للقدرة المضادة للالتهابات للمستخلص الميثانولي من *Gleditsia triacanthos*

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المُستَخلَص

تاريخ استلام البحث: 2020/10/22
تاريخ تعديل البحث: 2020/12/01
تاريخ قبول البحث: 2020/12/14

تحتوي النباتات على العديد من المكونات الكيميائية النباتية، والتي تقدم الكثير من الانشطة البيولوجية. لهذا الغرض، فإن الهدف الأساسي من هذه الدراسة هو تحديد التركيب الكيميائي النباتي لمستخلص القرون (ثمار) *Gleditsia triacanthos* ، والتي تستخدم بكثرة في الطب التقليدي. في الخطوة الثانية، تم تقييم النشاط المضاد للالتهابات لهذه المركبات في الجسم الحي على فئران ويستار.

أظهرت اختبارات الفحص الكيميائي النباتي أن مستخلص *Gleditsia triacanthos* غني بمجموعات قليلة بشكل رئيسي: العفص الكلي، والفلافونويد ، والكومارين ، والسابونوزيدات. لقد وجد أن المستخلص الميثانولي غني بشكل خاص بالمركبات الفينولية ، مع محتويات كبيرة من البوليفينول الكلي 7314. ± 512. ملغ EAG / جم ، الفلافونويد ؛ 659. ± 0.52 ملغ EQ / جم ، والعفص المكثف ؛ 956. ± 642. مجم EC / جم. أظهرت نتائج تقييم النشاط المضاد للالتهابات في الجسم الحي ، باستخدام المستخلص الميثانولي ، انخفاض في رد الفعل الالتهابي في نموذج تحريض ديكستران 1 ٪.و ذلك يتضح من نسبة تثبيط الالتهاب التي تتراوح من 6.56 إلى 10.76 ٪. تظهر الدراسة الحالية أن مستخلص ثمار *Gleditsia triacanthos* لها نشاط مضاد للالتهابات، ما يبرر استخدامها في الطب التقليدي. لذلك فهو ذو أهمية علاجية كبيرة كمركب بديل للوقاية من الالتهاب.

الكلمات الدالة: *Gleditsia triacanthos*، استخلاص، مركبات الفينول، نشاط مضاد للالتهابات.