Evaluation of the curative role, Anti-inflammatory and anti-oxidative activity of some dietary spices on carrageenan induced paw edema in albino Wistar rats

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ABSTRACT

Background: Inflammation is a response of the immune system, guarding the individual against infection. It is a major burning problem worldwide and billions of individuals are affected. Some of the spices used daily in Algerian cuisine have been known to possess major anti-inflammatory effects. Turmeric, red pepper, ginger, cinnamon and cumin etc. are a few of the wide spectrum of spices used in Algeria.

The aim of the study was to investigate the anti-inflammatory effect and anti activity of some dietary spices in carrageenan induced models of inflammation on Wistar rats.

Methods: Albino wistar rats were divided into four groups. Inflammation was induced on the animal by injecting the right hand paw with carrageenan (0.1 ml of 1%). Group 1 was fed with food and water and treated with carrageenan (control) whereas group 2 and 3 treated with different doses (100 and 200 mg/kg/bw) of aqueous extract of some dietary spices with carrageenan, respectively. Group 4 treated with standard drug dicyfenac sodium (10 mg/kg b.w., reference drug for inflammation), once for 24 hrs.

Results: The content of total phenolic was 137.2±21.6 mg/g and flavonoids 122.3±5.8 mg/g. After 60, 120, 180, 240 and 300 min, the data indicate that some dietary spices 100 and 200 mg/kg was significantly effective reducing paw edema volumes induced by carrageenan compared to control (p <0.01). The levels of serum pro-inflammatory white blood cells, neutrophile, lymphocyte, monocyte, eosinophile, total protein and protein c reactive and total cephalin KADN and platelets in the control group were significantly increased by carrageenan injection compared with the normal group. The rats pretreated with some spices 100 and 200 mg/kg had significantly lower levels of pro-inflammatory to compare control group. The paw edema in carrageenan-induced rats was considerably reduced by treating with 100 and 200 mg/kg aqueous extract of some dietary spices when compared to the untreated rats (p<0.001).

Conclusion: The present results clearly demonstrated that carrageenan significantly increased paw edema whereas some dietary treated rats significantly decreased the paw edema. The study underlines the importance of some of the frequently used spices (Turmeric, red pepper, ginger, cinnamon and cumin) in the treatment of inflammation.

Keywords: inflammation; herbal medicine; paw edema; spices, Algeria

INTRODUCTION

Inflammation is a major burning problem worldwide and billions of individuals are affected. It is well known, the herbal formulations had been used preventive and therapeutic medicine for a long time. Healing properties of medicinal plants were noted for ancient medical books. In traditional medicine, plants have long been used as alternative treatments for a wide range of diseases, including inflammatory processes of diverse origins, and have provided symptomatic relief comparable to that obtained from allopathic medicines.

Spices and herbs are usually whole plants or parts of plants that are used for flavouring and preservation of food all over the world. The people of the Algeria subcontinent exploit their rich biodiversity and use various plants or their parts as spices for daily cooking and preservation of food from...
time immemorial. These spices also have medicinal properties that have been underlined in the traditional systems of medicine like red pepper, ginger, cinnamon, etc.

Various plants/plant parts are used as spices in Algeria. Turmeric, red pepper, ginger, cinnamon and cumin etc. are a few of the wide spectrum of spices used in Algeria. Most of the spices have been studied for their various pharmacological properties. Cumin seed is known for its anti-microbial, anti-dabetic, anti-oxidant, anti-tumorigenic properties alongside its gastroprotective, hepatoprotective and cardioprotective properties 5.

There are various mechanisms to an inflammatory reaction, which can contribute to the associated symptoms and tissue injury. Edema, granuloma formation, and leukocyte infiltration signify such components of inflammation.

The carrageenan (Carr) induced edema, as an inflammation model, is usually used to assess the contribution of natural products to resist the biochemical changes associated with acute inflammation. When Carr is injected, acute inflammation with edema appears, along with a production of free radicals as well as a release of inflammatory mediators 4. The anti-inflammatory effect of some of the medicinal plants/herbs have been validated and few others disproved. The present study focuses on the anti-inflammatory and anti-activity potential of a few of the spices used in Algeria.

METHODS

Plant material

The various spices used in the Algerian household were randomly selected after consultation with the persons of the household. Turmeric, red pepper, ginger, cinnamon and cumin were collected during March-April 2019 from SBA vegetable market Algeria. The plant material was taxonomically identified by a botanist.

Test for flavonoids

To 1 ml of the extract, 5 ml of dilute ammonia is added. In addition, of 1 ml concentrated H2 SO4 a yellow color solution is temporarily produced which indicate the present of flavonoid. As a confirm test for flavonoid, a few drops of 3% aluminum solution is added to the plant extract and formation of a permanent yellow color solution indicates the presence of flavonoid. Test for saponins To 1 ml of extract, 1 ml of distilled water was added and shaken vigorously; a stable permanent form will develop indicating its presence. The result was positive for the test 5.

Test for phenols

A portion of the extract of the samples was diluted with distilled water in a ratio 1:4. Few drops of 10% ferric chloride solution were added. The appearance of green solution indicates the presence of phenol, which was present 6.

Experimental animals

Female rats of weighing 150-200 g were procured from the Pasteur Institute (Algiers, Algeria) and were housed in a clean polypropylene cages and maintained under standard laboratory conditions (temperature 25±2°C with dark/light cycle 12/12 hrs).

They were fed with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory conditions for 10 days before experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

2.2.3. Model of Carrageenan-Induced Paw Edema

Lambda carrageenan was purchased from Sigma Chemical Company (St. Louis, USA). It was used to induce paw edema. 1% w/v carrageenan was prepared by dissolving 1 mg of carrageenan in 100 mL of normal saline.

The anti-inflammatory activity was evaluated by the method of Carrinduced edema in the subplantar region of the right hind paw of the rats. Edema was induced by an injection of 0.1 mL of 1% freshly prepared suspension of carrageenan 7.

The immune system recognizes damaged or infected cells, pathogens, and begins the healing process itself. But, if the process is not regulated, or the offending agent persists, the intended protective process tends to be destructive 8.

Statistical analysis:

The statistical analysis is performed with IBM SPSS v 26 software. The graphical representation is illustrated with Microsoft Excel 2019, The results are expressed as mean and standard deviation. The comparison between the experimental groups is carried out with the one way ANOVA test, followed if significant by the Tukey post hoc test, Welch test or kruskal-Wallis test, The significance level is 0.05.

RESULTS

Preliminary, the presence of the phytochemicals was assessed to identify the potential constituents present in the extract. The aqueous extract of some diatery spices was also tested for the presence of phytochemicals by the methods described in the materials and methods section. Table 2 and figure 1, 2 show the presence of phytochemical constituents such as flavonoids and phenol in the aqueous extract of spices.

Total phenolic and flavonoids contents

The flavonoid contents of the extract in term of rutin equivalent (Standard curve equation: y = 0.153x + 0.005, R2 = 0.984 ), and the flavonoid content in the extract of some diatery species was 122.3±5.8 mg/g. The total content of phenolic compoundshowed as Gallic acid equivalent (Standard curve equation: y = 0.239x + 0.155, R2 = 0.997 ), and the total phenolic compound was 137.2± 21.6 mg/g in some diatery species.
Effect of some dietary spices on hind paw edema

Therefore the present study is undertaken in an aim to screen the efficacy of anti-inflammatory potential of some dietary spices in experimental rats. In the present study, the acute inflammation was experimentally induced by Carrageenan.

The different doses of some dietary spices treated rats showed an inhibition of Carrageenan induced paw edema in all observed time intervals as compared to Carrageenan induced paw edema.

Anti-inflammatory test

In subplantar injection of carrageenan induced a time-dependent increase in paw edema consisting of a relatively rapid early phase (up to 3 hour), (p = 0.003), and followed by late phase (4-5 hour). In the control group, the volume of hind paw edema was significantly increased by carrageenan injection in both phases and it reached peak levels at the late phase. Pretreatment of rats with some dietary spices (100 and 200 mg/kg) and DICLOFENAC (10 mg/kg) administered by oral resulted in inhibition (**p<0.001) of carrageenan-induced hind paw edema in both phases (FIGURE 3, 4).

Aqueous extract of spices at a dose of 200 mg/kg exhibited significant antiinflammatory activity in carrageenan induced rat paw oedema model (Figure 5).

The levels of serum pro-inflammatory white blood cells, neutrophile, lymphocyte, monocyte, eosinophile, total protein and protein c reactive and total cephalin KADIN and platelets in the control group were significantly increased by carrageenan injection compared with the normal group. The rats pretreated with some spices 100 and 200 mg/kg had significantly lower levels of pro-inflammatory to compare control group (Figure 6-15).

Therefore, the oral administration of some spices 200 mg/kg to pretreated group resulted in significant (**p<0.01) increase of red blood cells and hemoglobin and fibrinogen compared with the control group (Figure 16-18).
Figure 6: Variation of white blood cells count between the different groups

Figure 7: Variation of neutrophils count between the different groups

Figure 8: Variation of lymphocytes count between the different groups

Figure 9: Variation of monocytes count between the different groups

Figure 10: Variation of eosinophiles count between the different groups

Figure 11: Variation of basophiles count between the different groups
Figure 12: Variation of platelet count between the different groups

Figure 13: Variation of total proteins between the different groups

Figure 14: Variation of protein C reactive between the different groups

Figure 15: Variation of cephalin kaolin between the different groups

Figure 16: Variation of red blood cells count between the different groups

Figure 17: Variation of hemoglobin concentration between the different groups
DISCUSSION

A great number of spices possess anti-inflammatory activity though some like mustard is known for their pro-inflammatory activity too.

The anti-inflammatory effects of some of the medicinal plants have been validated and others disproved.

This investigation was the first experimental study designed to evaluate some dietary spices (combination of Turmeric, red pepper, ginger, cinnamon and cumin) would have anti-inflammatory effect on carrageenan a. From the results of the study, the some spices had sufficient potential to treat inflammatory disorders by reducing proinflammatory protein c reactive. The present study showed that the carrageenan-induced inflammation an elevation in the levels of serum neutrophil and eosinophil cells with hind paw edema. These observed results are the major symptoms of inflammatory conditions.

In the current study, it was observed that both phases of the carrageenan-induced hind paw edema were significantly reduced by pretreatment of rats with some spices.

The result of this study suggests that aqueous extract of some spices (Turmeric, red pepper, ginger, cinnamon and cumin) has anti-inflammatory effect comparable to those of the standard drugs such as DICLOFENAC. Carrageenan-induced inflammatory process is believed to be biphasic.

This observation is in line with a similar study carried out by Panahi et al.,2016 which the Curcumin, isolated from turmeric, has been widely studied for its anti-inflammatory activity. In persons with metabolic syndrome, treatment with curcumin inhibited inflammatory mediators and cytotoxins like tumour necrosis factor-alpha (TNFa), tumour growth factor-beta (TGF b), interleukin 6 (IL-6) and monocyte chemoattractant protein 1(MCP-1) 14. Edwards et al.(2017) referred curcumin as a pro-drug which on oxidative activation inhibited p65 subunit of nuclear factor kappa beta (NF-kb) and inhibitor kappa beta kinase (IKKb), the enzymes responsible for activation of NF-kb and its subsequent translocation to the nucleus to activate the pro-inflammatory mediators. The studies indicate that more than one component of turmeric is potent enough to be developed into an antiinflammatory drug.

Also, Both the extracts caused a significant reduction in paw edema induced by carrageenan in Wistar rats. The ethanolic extract was more effective as an analgesic as it reduced the number of writhes (in acetic acid induced writhing) and increased response time (in Eddy's hot plate method) while the aqueous extract did not show changes in the latter method.

The aqueous extract of ginger inhibited carrageenin induced paw edema in Sprague Dawley rats at doses of 25-200 mg/kg b.w. It also inhibited the inflammatory markers like NO, PGE2, inflammatory cytotoxins like TNFα, IL-1β, IL-6, IFN-γ, chemokines and increased the total antioxidant capacity as measured in inflammatory exudates obtained from carrageenan injected hind paws of rats. Significant histopathological changes from control group were observed in rats treated with 200 mg/kg b.w. ginger extract.

Cuminaldehyde, one main component obtained from cumin, and its bioconverted molecules also inhibited COX-2 and lipoxygenase activity, another well known mediator of inflammation as studied by in-silico molecular docking method.

Besides its use as spice, Black pepper is also used as pesticides, insecticide and in perfumery. Traditionally they were used to treat menstrual problems, ear-nose-throat related problems, diarrhoea, gastric problems, bed sore etc.

Piperine, an alkaloid obtained from black pepper, showed a dose dependent inhibition of TNFa, IL-1β, IL-6 and PGE2 production from BV-2 microglial cells at a dose of 25-100µg/ml. This reduction was due to inhibition in NF-κβ signalling pathway and upregulation of nuclear factor E2 related factor 2 (Nrf-2) and haem oxygenase-1(HO1) pathway.

Piperine brought down levels of NO, TNF-α, IL-1β, PGE2 along with the increase of IL10. It upregulated the anti-oxidant enzymes like glutathione (GSH), catalase and superoxide dismutase (SOD), reduced thiobarbituric acid reactive substances (TBARS), myeloperoxidase (MPO) and elastase activity along with the reduction in arthritic parameters as confirmed by histopathological studies such as decrease of cartilage erosion, absence of mononuclear infiltration in synovium etc.

CONCLUSION

In the current study, it was observed that both phases of the carrageenan-induced hind paw edema were significantly reduced by pretreatment of rats with some spices.

Consumption of spices in our daily life is associated with various health benefits, reduction of inflammation being one of them.

REFERENCES