Shaheen *et al.*
Bulletin of the National Research Centre (2022) 46:26
https://doi.org/10.1186/s42269-022-00708-0

Bulletin of the National Research Centre

RESEARCH

Open Access

Anti-inflammatory and analgesic activities of black cumin (BC, *Nigella sativa* L.) extracts in in vivo model systems

Nazma Shaheen[1*], Afiatul Azam[1], Amlan Ganguly[2], Saeed Anwar[3], Md. Sorwer Alam Parvez[3], Ujwal Punyamurtula[4] and Md. Kamrul Hasan[5,6]

Abstract

Background: Black cumin (*Nigella sativa*) is a widely used ingredient of traditional medicine for its broad-spectrum pharmacological actions, including analgesic, bronchial asthma, anti-inflammatory properties, and others. We sought to evaluate BC extracts' efficacy for the anti-inflammatory and analgesic properties using a comprehensive in vivo and in silico experimental setup. To investigate whether BC extract has anti-inflammatory and analgesic therapeutic potentials in vivo anti-inflammatory activity by carrageenan-induced rat paw edema, analgesic activity by acetic acid-induced writhing test and ingenuity analysis of the BC extracts in inflammation control.

Results: The acetic acid-induced writhing test had shown a dose-dependent reduction of writhing number following BC administration. Rat paw edema test showed the dose-dependent reduction of paw edema volume following BC administration. Ingenuity Pathway Analysis (IPA) suggested BC extracts containing ferulic acid, p-coumaric acid, kaempferol, and quercetin can inhibit inflammation.

Conclusions: This study suggests that bioactive compounds in BC extract act as an anti-inflammatory and analgesic agent by regulating several downstream and upstream inflammation pathways.

Keywords: Black cumin, BC extract, *Nigella sativa*, Anti-inflammation, Analgesic, Edema

Background

Inflammation is a fundamental part of the body's physiological defense mechanisms against pathogenic infections and toxic substances (Medzhitov 2008). It is involved in the body's response to both the initial cause and the consequences of an injury. Often, however, inflammations can be triggered inappropriately, leading to tissue destruction, which in turn can result in a range of inflammatory disorders, including rheumatoid and gouty arthritis, psoriasis, and Crohn's disease (Okin and Medzhitov 2012). Many studies suggest that a persistent inflammatory condition can be pervasive and develop into more clinically severe afflictions, including cardiovascular disease and cancer, often with fatal outcomes (Roifman et al. 2011; Okin and Medzhitov 2012; Laveti et al. 2013).

Current management strategies for inflammatory diseases include medications, relaxation, exercise, and surgery to correct joint damage. The medications used to treat inflammatory diseases, such as non-steroidal anti-inflammatory drugs, corticosteroids, cyclophosphamide, hydroxy-chloroquine, and biologic drugs, possibly minimize disease progression by lowering joint pain and swelling and the inflammation itself (Diaz-Borjon et al. 2006; Cheng 2012; De Mattos et al. 2015; Cavaillon and Singer 2018). The management strategy is contingent on many factors, including the patient's age, medical background and comorbidity, immunity status, and the severity of inflammatory disease symptoms (Cheng

*Correspondence: nazmashaheen@du.ac.bd; nzmshaheen58@gmail.com
[1] Institute of Nutrition and Food Science, University of Dhaka, Dhaka 1000, Bangladesh
Full list of author information is available at the end of the article

2012; Cavaillon and Singer 2018). However, the efficacy of these management strategies can be questionable, and even if the efficacy is satisfactory, many of these strategies are often not suitable for all patients because of the associated side effects (Li et al. 2017). Both steroidal and non-steroidal inflammatory drugs are associated with a high range of adverse effects in the long term (Hanania et al. 1995; Gor and Saksena 2011). There is thus a constant push toward developing an effective and curative therapeutic strategy to treat inflammatory diseases (Schwartz et al. 2017).

Medicinal plants are an essential source of bioactive compounds with potential therapeutic efficacy (Atanasov et al. 2015). Pharmacological investigations of medicinal plants can yield primers for the effective treatment of inflammatory diseases (Atanasov et al. 2015). Black Cumin (BC, *Nigella sativa* L.) is a well-known medicinal plant used extensively in Unani, Ayurvedic, and Siddhi medicine for centuries (Houghton et al. 1995). This herb, endemic to South Asian and Mediterranean countries, is rich in bioactive compounds, including tocopherols, alkaloids, saponins, and vitamins A and C, all of which contribute to its biological functionality (Butt and Sultan 2010). Overwhelming evidence indicates the presence of bioactive ingredients in BC that can counteract the underlying pathophysiology of many diseases, including cancers, inflammatory conditions, cardiovascular defects, autoimmune disorders (Butt and Sultan 2010; Dajani et al. 2016; Tavakkoli et al. 2017). Previous studies have highlighted BC's potential anti-inflammatory and analgesic activities (Pise and Padwal 2017; Ikhsan et al. 2018). However, only a few studies have revealed BC's anti-inflammatory effects on subacute and chronic models of inflammation (Pise and Padwal 2017).

In this study, we sought to investigate the dose–response effects of the anti-inflammatory activity of BC in rats and mice with in vivo anti-inflammatory, and analgesic activities. In addition, the present study attempted to develop a probable mechanism of action for *Nigella sativa* through Ingenuity pathway analysis.

Methods

Collection and preparation of black cumin samples

Trained food sample collectors collected the BC seed samples from Newmarket Kancha Bazar, Dhaka, Bangladesh. The BC seed samples were then critically checked by the expert faculty member of the Department of Botany, University of Dhaka. Preparation and processing of the collected samples were done using standard operating procedures (Bashir et al. 2015). Processing involved drying the fresh samples at 25–30 °C, grinding them by an electric blender, and preserving them in an airtight container until analysis.

Extraction procedure

Multiple rounds of sequential extraction (initially by hexane/dichloromethane (1:1 v/v) (Merck, Germany; hexane-296090, dichloromethane-270997) and then by AWA (acetone/water/acetic acid 70:29.5:0.5) (Merck, Germany; acetone-650501, acetic acid-A16283) was performed in an accelerated solvent extraction equipment known as ASE 200 (DIONEX, USA, catalog number: 055422) (Mamun et al. 2012). A detailed description of the extraction procedure has been reported elsewhere (Mamun et al. 2012; Shaheen et al. 2016). Dried extract of AWA was dissolved to make DMSO dissolution for the administration of the experimental animals (Shaheen et al. 2016).

Experimental animal

Swiss Albino mice (Scientific name: *Mus musculus*) (5–6 weeks of age; 20–30 g) and Long-Evans rats (Scientific name: *Rattus norvegicus*) (7–8 weeks of age; 100–130 g) were collected from the Animal Research Branch, International Centre for Diarrheal Disease Research, Bangladesh (ICDDR, B). The animals were housed in polyvinyl cages for seven days and maintained under standard laboratory conditions (temperature 25 ± 2 °C) and 12 h light–12 h dark cycles. The animals were fed on a standard laboratory animal diet formulated by ICDDR, B, and water ad libitum. To keep the hydration rate constant, food and water were stopped 12 h before the experiments. The Ethical and Animal Care Committee of the Institute of Nutrition and Food Sciences, University of Dhaka, Bangladesh, critically reviewed and approved this study involving in vivo models. The procedures described in this study were conducted in accordance with the Bangladesh Biosafety and Biosecurity guidelines 2019 and institutional oversight performed by qualified veterinarians (2019).

Assessment of anti-inflammatory activity by carrageenan-induced rat paw edema

Evaluation of anti-inflammatory activity was conducted in vivo in the Long Evans rat model. At first, 200 mg of samples (i.e., dried AWA extract) were mixed with 5.0 mL of DMSO in a shaking incubator at 130 rpm overnight. Then the mixture was centrifuged at 3000 rpm for 5.0 min. Then the supernatant was collected and pipetted 500 µL of aliquots to store at 25 °C.

To determine anti-inflammatory activity, edema was induced by the phlogistic agent carrageenan (Winter et al. 1962b). The control group (n = 3) received normal saline (p.o.) (10 mL/kg), while group 2 received a standard drug, Diclofenac sodium (50 mg/kg, p.o.). The rest of the groups were given 800 (n = 3), 400 (n = 3),

and 200 mg/kg body weight (n = 3) p.o. of extract preparations. Thirty minutes after administering sample extracts, each rat received 0.1 mL of 1% (w/v) carrageenan (injected into the sub-plantar region of the right hind paw subcutaneously) (Sigma Aldrich, # C-1013, Germany). Paw volumes of the right hind paw of each rat were measured by plethysmometer before and 1, 2, 3, and 4 h after carrageenan injection to determine the edema volume. The hind paw volume was evaluated for anti-inflammatory activity and expressed as % inhibition of the hind paw volume, which was calculated by the following equation (Winter et al. 1962b):

$$\% \text{ inhibition} = [(Vc - Vt)/Vc] \times 100$$

Here, Vc = average paw volume of the control group, and Vt = Average paw volume of the treated group.

Assessment of analgesic activity by acetic acid-induced writhing test

Peripheral analgesic activity of the extracts was determined by the acetic-acid-induced writhing inhibition method in mice (Whittle 1964). The control group (n = 3) (group 1) received normal saline (10 mL/kg, p.o.). Group 2 received a reference drug, Diclofenac sodium (50 mg/kg, p.o.). The rest of the groups received 800 (n = 3), 400 (n = 3), and 200 mg/kg body weight (n = 3) p.o. of dried AWA sample extracts. Each mouse was administered intraperitoneally after 30 min of treatment with 0.6% acetic acid (10 mL/kg). Later, the writhing numbers of each mouse were observed for 10 min. To evaluate the level of analgesia, writhing numbers of the sample treated mice were compared with the writhing numbers of the control groups. Percent-inhibition of writhing was calculated using the following equation:

$$\% \text{ inhibition of writhing} = [(Wc - Wt)/Wc] \times 100$$

Here, Wc = Average number of the writhing of the control group, and Wt = Average number of the writhing of the treated group.

Ingenuity analysis of the BC extracts in inflammation control

We evaluated the effects of the compounds extracted from BC using the knowledge-based path explorer feature of the Ingenuity Pathway Analysis (IPA) tool (Chindelevitch et al. 2012; Krämer et al. 2014). We explored new ingenuity-based pathways that showed the relationship between the genes involved in the biosynthetic pathways of inflammation-related compounds (e.g., arachidonic acids, prostaglandins, and leukotrienes) and compounds in the BC extracts, which includes ferulic acid, quercetin, p-coumaric acid, and kaempferol. The shortest possible pathways were generated individually for each compound where the compounds in the BC extracts were set as the initial compound, and inflammation-related compounds were selected as the target.

Statistical analysis

The data obtained were expressed as mean ± SEM. Statistically significant differences between groups were calculated by the application of analysis of variance (one-way ANOVA) followed by Dunnett's test. P values less than 0.05 ($p < 0.05$) were used as the significance level.

Results

In vivo anti-inflammatory activity

4 h after carrageenan injection, the maximum edema volume in control Long Evans adult rat models was 1.18 ± 0.03 ml. Another group of rats pretreated with Diclofenac sodium (reference drug; 50 mg/kg, p.o.) showed significantly reduced ($p < 0.05$) edema in the paw. Dose–response analysis of DMSO extract of BC produced a significant inhibition of paw edema in Long Evans adult rats in a dose-dependent manner at 400, and 800 mg/kg, p.o., administration of carrageenan ($p < 0.05$) (Table 1).

The maximum inhibition of rat paw edema by BC was noted 4 h after carrageenan administration at a dose of 800 mg/kg, p.o., as compared to the control but less than the reference drug. Figure 1 shows the changes of percent inhibition of carrageenan-induced paw edema volume at 1, 2, 3, and 4 h at the doses of 800 mg/kg, p.o., by the DMSO extract of BC when compared to the control. We found a slightly increasing trend from hour 1 to hour 2, a slightly decreasing trend in hour 2 to hour 3, and a relatively increasing trend from hour 3 to hour 4.

In vivo analgesic activity

The effects of DMSO extract of BC on 0.6% acetic acid-induced writhing in Swiss albino mice are summarized in Table 2. A dose-dependent and significant ($p < 0.05$) reduction in the number of abdominal constrictions induced by intraperitoneal administration of 0.6% acetic acid was observed with oral administration of BC, at the doses of 400, and 800 mg/kg, p.o., when compared to the control. Among the different doses, the DMSO extract of BC at the dose of 800 mg/kg exhibited the maximum inhibition of the number of writhing compared to control and followed a dose-dependent inhibition trend in two other doses, 200 and 400 mg/kg, p.o.

The maximum inhibition of rat paw edema by BC was noted 4 h after carrageenan administration at a dose of 800 mg/kg, p.o. Compared to the control but less than the reference drug. Figure 1 shows the percent decrease in carrageenan-induced paw edema volume changes at

Table 1 Effects Black Cumin extracts by DMSO extracts of *BC* on carrageenan-induced paw in rats

Group	Dose (mg/kg, p.o.)	Paw edema volume (ml) (% of inhibition)			
		1 h	2 h	3 h	4 h
Control	–	0.83 ± 0.02	0.96 ± 0.02	1.07 ± 0.02	1.18 ± 0.03
Diclofenac sodium	50	0.44 ± 0.0*	0.53 ± 0.02*	0.60 ± 0.01*	0.66 ± 0.01*
		47.18	45.32	44.10	43.79
	200	0.71 ± 0.01*	0.78 ± 0.01*	0.89 ± 0.02*	0.94 ± 0.03*
		14.15	19.03	16.26	20.62
Nigella sativa	400	0.63 ± 0.001*	0.74 ± 0.01*	0.83 ± 0.001*	0.85 ± 0.001*
		23.39	23.52	21.88	27.69
	800	0.61 ± 0.01*	0.70 ± 0.01*	0.81 ± 0.01*	0.83 ± 0.01*
		26.21	26.99	24.06	29.66

[a] Presented as mean ± SEM (n = 3)

[*] $p < 0.05$ compared with the control group (Dunnett's test)

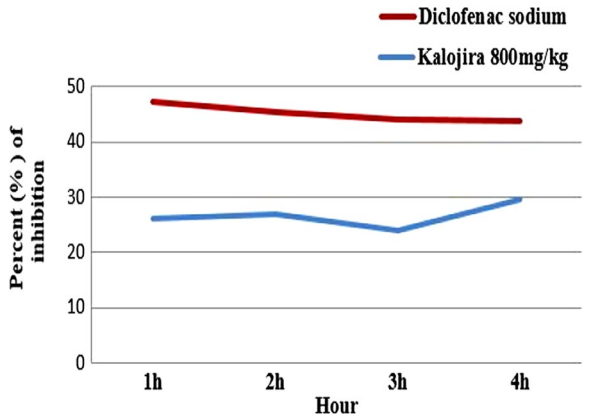

Fig. 1 Time course in the changes of percent inhibition on carrageenan-induced paw edema volume of BC at 1, 2, 3, and 4 h at the dose of 800 mg/kg, p.o.

Table 2 Effect of Black Cumin extracts by DMSO on acetic acid-induced (0.6%) writhing in mice

Group	Dose (mg/kg, p.o.)	No. of writhing[a]	% inhibition
Control	–	23.67 ± 1.2	–
Diclofenac sodium	50	14.70 ± .89**	37.89
	200	18.01 ± 1.0*	23.91
Nigella sativa	400	17.35 ± 0.67*	26.65
	800	16.62 ± 0.33*	29.78

[a] Presented as mean ± SEM (n = 3)

[*] $p < 0.05$ compared with the control group (Dunnett's test)

[**] $p < 0.001$ compared with the control group (Dunnett's test)

first, second, third, and fourth hours post-administration of 800 mg/kg, p.o., of DMSO extract of BC when compared to the control.

In silico analysis provides insights into the mechanism of how BC extracts reduce inflammation

The *in-silico* analysis of the bioactive compounds of BC illustrates a facet reactome depicting potential models of how BC extracts reduce inflammation in humans (Fig. 2, Additional file 1: Table S1). Both prostaglandin and leukotriene synthesize from the arachidonic acid. Quercetin, p-coumaric acid, and kaempferol reduce the expression of myc, MAPK, EGFR, and TNF, all of which are involved in the upregulation of the expression of arachidonic acid. Consequently, quercetin, p-coumaric acid, and kaempferol downregulate the expression of arachidonic acids.

Ferulic acid, another component in the BC extract, negatively induces the synthesis of phospholipids. Phospholipid biosynthesis is critically important for the synthesis of arachidonic acids. Also, ferulic acids upregulate the expression of HMOX and nrf2, resulting in the reduction of prostaglandin E2 (PGE2). Moreover, all the compounds of the *Nigella* sativa extract directly reduce the expression of cox1 and cox2, two essential factors for the synthesis of PGE2. Collectively, ferulic acid, quercetin, p-coumaric acid, and kaempferol help reduce inflammation by interfering with the extended arachidonic acid pathway (Fig. 2; Additional file 1: Table S1).

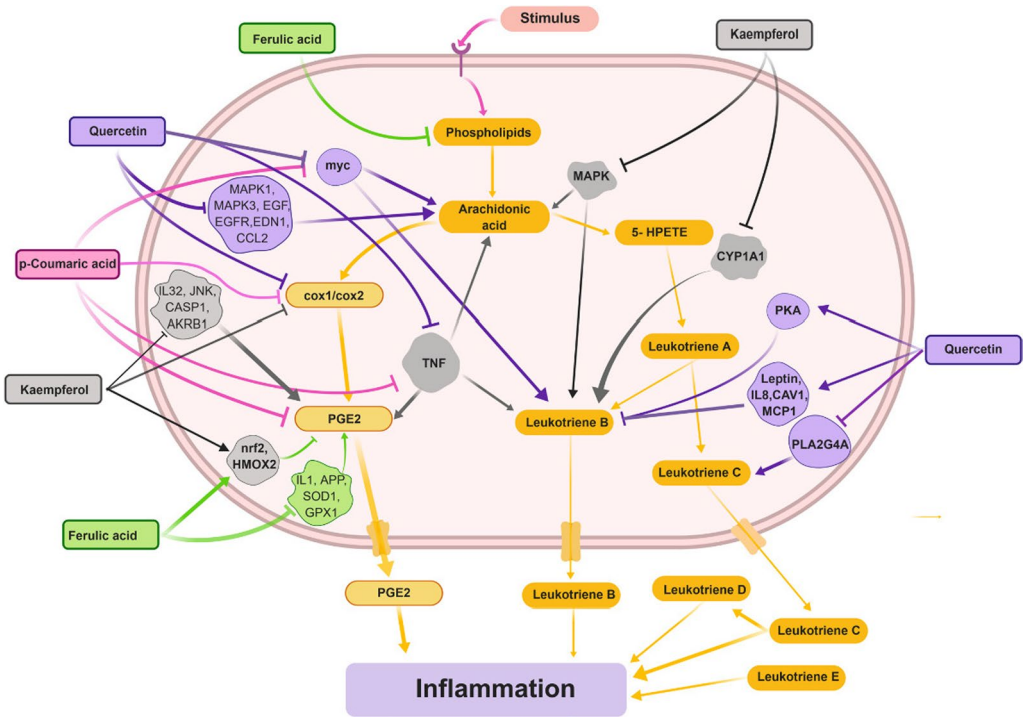

Fig. 2 Possible mechanism of action of BC extracts to reduce inflammation in humans. In the BC extracts, phenols and polyphenols, e.g., ferulic acid, p-coumaric acid, kaempferol, and quercetin, inhibit the signaling molecules, stimulation of which may directly or indirectly result in inflammation. Proteins linked to inflammation participate in several canonical pathways underlying a range of biological activities. Ingenuity pathway analysis (IPA) identified that the BC extracts' components could target many of these proteins (Additional file 1: Table S1) and control inflammation. Arrows (↓) indicate direct stimulatory modification, whereas light-up signs (⊤) indicate an inhibitory modification. The names of the proteins followed the Hugo nomenclature, NCBI Entrez, UniProtKB/Swiss-Prot system

Discussion

In this study, we demonstrate the in vivo dose–response of the AWA extracts with DMSO to evaluate the anti-inflammatory and analgesic activities in the experimental models (Winter et al. 1962a). Carrageenan-induced paw edema in rat models is increasingly being employed to determine anti-inflammatory effects in animal models. In response to carrageenan injection, edema formation occurs in a biphasic process. Mast cells around the damaged tissue released prostaglandin with histamine and serotonin mediate the first phase (1–2 h) of the carrageenan model. Bradykinin, leukotrienes, polymorphonuclear cells, and continuous secretion of prostaglandins from macrophages tissue mediate the second phase (3–5 h) of the carrageenan model (Winter et al. 1962b; Rosa et al. 1971). Our study indicates that the DMSO extracts of BC exhibited significant ($p < 0.05$) inhibition of paw edema in rats at the doses of 400, and 800 mg/kg, p.o., in the second phases of inflammatory response. DMSO extracts of BC at the 800 mg/kg dose produced the maximum inhibition of carrageenan-induced paw edema volume and inhibition is also seen in other doses in a dose-dependent manner. The present study showed the maximum inhibition of carrageenan-induced paw edema volume in rats by BC in the second phase of post carrageenan injection at 3–5 h, maybe due to the modulatory principles acting with the prostaglandin alley. Moreover, the present study supports the previous findings that BC seed polyphenol plays a crucial role as a protective factor against inflammation (Shaheen et al. 2016).

The "acetic acid-induced writhing experiment" is a well-known protocol for assessing the analgesic potency of medicinal products (Afzal et al. 2012). Intraperitoneal injection of acetic acid that triggers pain sensation is due to the prostaglandins and lipoxygenase products from arachidonic acid liberated from phospholipids by cyclooxygenase (Fish et al. 2008). Thus, the significant ($p < 0.05$) reduction of writhing in this study by DMSO extracts of BC at the doses of 400, and 800 mg/kg, p.o., suggest analgesic activity peripherally mediated through inhibition of prostaglandins and other endogenous pain mediators. The highest concentration (800 mg/kg) of the test samples exhibited a peak of analgesic effect significantly ($p < 0.05$) in the acetic acid-induced writhing test. For other doses, reduction of writhing were found in a dose-dependent manner. These results indicate the

peripheral analgesic potential of the DMSO extracts of the test samples, which could be exhibited due to the suppression of peritoneal surface receptors through inhibited cyclooxygenase activity. The current study findings concordance with previous findings that BC seed polyphenol inhibits acetic acid-induced writhing in the mouse model (Ghannadi et al. 2005). We also note how to present findings that confirm previous work in the field by examining the literature. Specifically, the in vivo experiments performed in this study are in good agreement with those of prior experiments on BC's anti-inflammatory activity, lending credence to the idea that BC is a potent medicinal plant for therapeutic uses.

In addition to the wet-lab analyses, ingenuity pathway analysis provides evidence for the role of the *Nigella sativa* extracts in reducing inflammation. Earlier studies revealed that some molecules, including myc, MAPK, EGFR, TNF, are involved in the cellular responses for inducing the inflammation (Kaminska 2005; Bradley 2008; Sipos et al. 2016; Rayego-Mateos et al. 2018). We have found that compounds in the *Nigella* sativa extracts, including ferulic acid, quercetin, p-coumaric acid, and kaempferol, downregulate the inflammation-inducing signaling molecules in humans (Fig. 2). Many research groups have reported the potential role of activating pKA, HMOX, and nrf2 pathways, leading to anti-inflammatory effects (Alcaraz et al. 2005; Campo et al. 2012b, 2012a; Tu et al. 2019). Our analysis uncovered that compounds in the *Nigella* sativa extracts upregulate the pKA, HMOX, and nrf2 (Fig. 2).

Furthermore, we have shown the potential aspects of the anti-inflammatory mechanism of BC that were previously only speculated upon. For example, Bordoni et al. (2019) found BC to reduce only the acute inflammation suggested that BC's mechanism of action is tied to interleukins, TNF, and prostaglandins (Cinzia et al. 2013). In the ingenuity pathway analysis, we have shown that various components of BC act through IL32, IL8, IL1, TNF, and PGE2 to reduce inflammation. In together, ferulic acid, quercetin, p-coumaric acid, and kaempferol help reducing inflammation by interfering with the extended arachidonic acid pathway. In another study, researchers discovered that BC could decrease IL-6 levels or IL-1B levels, depending on storage conditions (Bordoni et al. 2019). These findings establish a connection between BC and interleukins in BC's anti-inflammatory activity, which we have at least partially outlined in Fig. 2.

Similarly, other researchers speculated that BC WSE inhibits COX2 and that BC inhibits prostaglandin synthesis through interaction with COX-1 and COX-2 (Babar et al. 2019). Both hypotheses are supported by the results of our Ingenuity pathway analysis, as depicted in Fig. 2. Furthermore, Babar et al. suggested that phenols/polyphenols in BC might be responsible for its anti-inflammatory activity (Babar et al. 2019). Our pathway analysis found that these phenols and polyphenols are ferulic acids, p-coumaric acid, kaempferol, and quercetin. It is speculated that the inhibitory effect on the secretion of leukotrienes and prostaglandins by thymoquinone may be responsible for BC's anti-inflammatory activity (Bashir et al. 2015). We have shown that this is the case, although, instead of thymoquinone, quercetin and ferulic acid were found to be the components of BC responsible for this aspect of its anti-inflammatory mechanism. In another study, *Nigella sativa* showed superior activity in screening *Nigella* species for in vitro inhibition of PGE2 production catalyzed by COX1 and COX2 (Landa et al. 2009). This lends credence to the mechanistic schematic we have depicted in Fig. 2; as shown in that diagram, several components of BC act to inhibit COX1/COX2 activity, which is necessary to produce PGE2. In yet another study, researchers discovered that levels of erk are decreased upon *Nigella sativa* treatment of damaged rat lungs (Aslan et al. 2015). The erk repression discussed here is supported by our mechanistic analysis, which has found that kaempferol, a component of *Nigella sativa*, inhibits ERK1/2 activity. As evidenced by these examples from the literature, the results we have found build on previous research to provide crucial insight into the medicinal properties of a common natural product.

Together with the in vivo studies and in silico analysis, this study provides strong evidence that the bioactive compounds in BC extract act as an anti-inflammatory agent by regulating several downstream and upstream inflammation pathways.

Conclusions

In summary, the present study's findings support that BC extract has anti-inflammatory, analgesic activity and in silico analyses suggested that the phenolic compounds in BC extracts may play a key role in inhibiting human inflammatory reactions. Thus, in understanding how to present findings related to prior work in the field, we have gained insight into the vitality of this research for future efforts to study the anti-inflammatory effects of *Nigella sativa*. Future studies should focus on elucidating the mechanistic pathways through which compounds in BC extracts exert such pharmacologic effects to prevent inflammation.

Abbreviations

BC: Black cumin; RBL: Rat basophilic Leukemia; IPA: Ingenuity pathway analysis; AWA: Acetone–water-acetic acid; DMSO: Dimethyl sulfoxide.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42269-022-00708-0.

Additional file 1. Components of the reactome Nigella sativa works on to impact on human inflammations.

Acknowledgements
We thank Aftab Uz Zaman Noor, MSc (Université Claude Bernard Lyon 1, France) for his in-kind assistance in conducting the in silico study.

Authors' contributions
Conceptualization, NS, MKH and SA; methodology, NS, AA, AG, SA, MKH and MSAP; software, SA and MSAP; validation and scrutinization, NS, MKH and UP; investigation, NS, AA, AG, SA and MSAP; formal analysis, NS, AA, AG, SA and MSAP; writing—original draft preparation, AA, AG and SA; writing—review and editing, NS, MKH and UP; supervision, NS; project administration, NS and MKH. All authors have read and approved the manuscript.

Funding
The author(s) received no specific funding for this project.

Availability of data and materials
Derived data supporting the findings of this study are available from the corresponding author [NS] on request.

Declarations

Ethics approval and consent to participate
All procedures were reviewed and approved by the Ethical and Animal Care Committee of the Biological Science faculty, University of Dhaka, Bangladesh and the Helsinki Declaration in 1975 (revised in 2000). The committee's reference number is not currently available.

Consent for publication
Not applicable.

Competing interests
The authors do not have any competing interests.

Author details
[1]Institute of Nutrition and Food Science, University of Dhaka, Dhaka 1000, Bangladesh. [2]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh. [3]Department of Genetic Engineering and Biotechnology, School of Life Sciences, Shahjalal University of Science and Technology, Sylhet 3114, Bangladesh. [4]COBRE Center for Cancer Research Development, Proteomics Core Facility, Rhode Island Hospital, Providence, RI 02903, USA. [5]Department of Biochemistry and Molecular Biology, Tejgaon College, National University, Gazipur 1704, Bangladesh. [6]Department of Public Health, North South University, Dhaka 1229, Bangladesh.

Received: 17 December 2021 Accepted: 11 January 2022
Published online: 02 February 2022

References

Afzal M, Gupta G, Kazmi I, Rahman M, Afzal O, Alam J, Hakeem KR, Pravez M, Gupta R, Anwar F (2012) Anti-inflammatory and analgesic potential of a novel steroidal derivative from *Bryophyllum pinnatum*. Fitoterapia 83:853–858. https://doi.org/10.1016/j.fitote.2012.03.013

Alcaraz M, Fernandez P, Guillen M (2005) Anti-inflammatory actions of the heme oxygenase-1 pathway. Curr Pharm Des 9:2541–2551. https://doi.org/10.2174/1381612033453749

Aslan A, Boydak D, Can MI, Kuloglu T (2015) Nigella sativa improves the carbon tetrachloride-induced lung damage in rats through repression of erk/akt pathway. Bangladesh J Pharmacol 10:654–659. https://doi.org/10.3329/bjp.v10i3.23486

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Babar ZM, Jaswir I, Tareq AM, Ali Reza ASM, Azizi WM, Hafidz M, Ahfter F, Hasan M, Farhad S, Uddin MMR, Ichwan SJ, Ahmed QU, Taher M, Mawa I (2019) In vivo anxiolytic and in vitro anti-inflammatory activities of water-soluble extract (WSE) of *Nigella sativa* (L.) seeds. Nat Prod Res 35:2793–2798. https://doi.org/10.1080/14786419.2019.1667348

Bangladesh Biosafety and Biosecurity Guidelines for Handling and Disposal of Biohazardous Materials First Edition Bangladesh Biosafety and Biosecurity Society (2019) https://bdbiosafetysecurity.org/uploads/documents/Bangladesh_Biosafety_and_Biosecurity_Guidelines.pdf

Bashir MU, Qureshi HJ, Saleem T (2015) Comparison of anti-inflammatory activity Nigella sativa and diclofenac sodium in albino rats. J Ayub Med Coll Abbottabad 27:523–526.

Bordoni L, Fedeli D, Nasuti C, Maggi F, Papa F, Wabitsch M, De Caterina R, Gabbianelli R (2019) Antioxidant and anti-inflammatory properties of nigella sativa oil in human pre-adipocytes. Antioxidants. https://doi.org/10.3390/antiox8020051

Bradley JR (2008) TNF-mediated inflammatory disease. J. Pathol. 214:149–160. https://doi.org/10.1002/path.2287

Butt MS, Sultan MT (2010) *Nigella sativa*: reduces the risk of various maladies. Crit Rev Food Sci Nutr 50:654–665. https://doi.org/10.1080/10408390902768797

Campo GM, Avenoso A, D'Ascola A, Prestipino V, Scuruchi M, Nastasi G, Calatroni A, Campo S (2012a) Protein kinase a mediated anti-inflammatory effects exerted by adenosine treatment in mouse chondrocytes stimulated with IL-1β. BioFactors 38:429–439. https://doi.org/10.1002/biof.1040

Campo GM, Avenoso A, D'Ascola A, Scuruchi M, Prestipino V, Nastasi G, Calatroni A, Campo S (2012b) The inhibition of hyaluronan degradation reduced pro-inflammatory cytokines in mouse synovial fibroblasts subjected to collagen-induced arthritis. J Cell Biochem 113:1852–1867. https://doi.org/10.1002/jcb.24054

Cavaillon J-M, Singer M (2018) Inflammation : from molecular and cellular mechanisms to the clinic. https://www.wiley.com/enus/Inflammation:+From+Molecular+and+Cellular+Mechanisms+to+the+Clinic,+4+Volume+Set-p-9783527338993.

Cheng H (2012) Genetic variation in resistance to inflammation and infectious disease. In: Inflammatory diseases-immunopathology, clinical and pharmacological bases. InTechOpen. https://doi.org/10.5772/26969

Chindelevitch L, Ziemek D, Enayetallah A, Randhawa R, Sidders B, Brockel C, Huang ES (2012) Causal reasoning on biological networks: interpreting transcriptional changes. Bioinformatics 28:1114–1121. https://doi.org/10.1093/bioinformatics/bts090

Cinzia N, Manuel C, Donatella F, Rosita G, Antonio DS, Cerasa LS, Isabel S, Valentina D, Roberto C (2013) Effects of early life permethrin exposure on spatial working memory and on monoamine levels in different brain areas of pre-senescent rats. Toxicology 303:162–168. https://doi.org/10.1016/j.tox.2012.09.016

Dajani EZ, Shahwan TG, Dajan NE (2016) Overview of the preclinical pharmacological properties of *Nigella sativa* (black seeds): a complementary drug with historical and clinical significance. J Physiol Pharmacol 67:801–817.

Diaz-Borjon A, Weyand CM, Goronzy JJ (2006) Treatment of chronic inflammatory diseases with biologic agents: opportunities and risks for the elderly. Exp. Gerontol. 41:1250–1255. https://doi.org/10.1016/j.exger.2006.10.010

Fish R, Danneman P, Brown M, Karas A (2008) Anesthesia and analgesia in laboratory animals. Elsevier Inc. https://www.elsevier.com/books/anesthesia-and-analgesia-in-laboratory-animals/fish/978-0-12-373898-1.

Ghannadi A, Hajhashemi V, Jafarabadi H (2005) An investigation of the analgesic and anti-inflammatory effects of *Nigella sativa* seed polyphenols. J Med Food 8:488–493. https://doi.org/10.1089/jmf.2005.8.488

Gor AP, Saksena M (2011) Adverse drug reactions of nonsteroidal anti-inflammatory drugs in orthopedic patients. J Pharmacol Pharmacother 2:26–29. https://doi.org/10.4103/0976-500X.77104

Hanania NA, Chapman KR, Kesten S (1995) Adverse effects of inhaled corticosteroids. Am. J. Med. 98:196–208. https://doi.org/10.1016/S0002-9343(99)80404-5
Houghton PJ, Zarka R, De Las HB, Hoult JRS (1995) Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 61:33–36. https://doi.org/10.1055/s-2006-957994
Ikhsan M, Hiedayati N, Maeyama K, Nurwidya F (2018) *Nigella sativa* as an anti-inflammatory agent in asthma. BMC Res Notes 11:744. https://doi.org/10.1186/s13104-018-3858-8
Kaminska B (2005) MAPK signalling pathways as molecular targets for anti-inflammatory therapy: from molecular mechanisms to therapeutic benefits. In: Biochimica et Biophysica Acta: Proteins and Proteomics. Biochim Biophys Acta, pp 253–262. https://doi.org/10.1016/j.bbapap.2005.08.017
Krämer A, Green J, Pollard J, Tugendreich S (2014) Causal analysis approaches in ingenuity pathway analysis. Bioinformatics 30:523–530. https://doi.org/10.1093/bioinformatics/btt703
Landa P, Marsik P, Havlik J, Kloucek P, Vanek T, Kokoska L (2009) Evaluation of antimicrobial and anti-inflammatory activities of seed extracts from six Nigella species. J Med Food 12:408–415. https://doi.org/10.1089/jmf.2007.0600
Laveti D, Kumar M, Hemalatha R, Sistla R, Naidu VGM, Talla V, Verma V, Kaur N, Nagpal R (2013) Anti-inflammatory treatments for chronic diseases: a review. Inflamm Allergy Drug Targets 12:349–361. https://doi.org/10.2174/18715281113129990053.
Li P, Zheng Y, Chen X (2017) Drugs for autoimmune inflammatory diseases: from small molecule compounds to anti-TNF biologics. Front Pharmacol. https://doi.org/10.3389/fphar.2017.00460
Mamun S, Shaheen N, Basak TA, Modiduzzaman MD, Banu CP, Takano-Ishikawa Y (2012) Hydrophilic antioxidant capacities and total phenol content of seasonal fruits of Bangladesh. Malays J Nutr 18:355–362.
De Mattos BRR, Garcia MPG, Nogueira JB, Paiatto LN, Albuquerque CG, Souza CL, Fernandes LGR, Tamashiro WMDSC, Simioni PU (2015) Inflammatory bowel disease: an overview of immune mechanisms and biological treatments. Mediators Inflamm. https://doi.org/10.1155/2015/493012
Medzhitov R (2008) Origin and physiological roles of inflammation. Nature 454:428–435. https://doi.org/10.1038/nature07201
Okin D, Medzhitov R (2012) Evolution of inflammatory diseases. Curr Biol. https://doi.org/10.1016/j.cub.2012.07.029
Pise HN, Padwal SL (2017) Evaluation of anti-inflammatory activity of nigella sativa: an experimental study. Natl J Physiol Pharm Pharmacol 7:707–711. https://doi.org/10.5455/njppp.2017.7.0204705032017
Rayego-Mateos S, Rodrigues-Diez R, Morgado-Pascual JL, Valentijn F, Valdivielso JM, Goldschmeding R, Ruiz-Ortega M (2018) Role of epidermal growth factor receptor (EGFR) and its ligands in kidney inflammation and damage. Mediat Inflamm. https://doi.org/10.1155/2018/8739473
Roifman I, Beck PL, Anderson TJ, Eisenberg MJ, Genest J (2011) Chronic inflammatory diseases and cardiovascular risk: a systematic review. Can. J. Cardiol. 27:174–182. https://doi.org/10.1016/j.cjca.2010.12.040
Rosa MD, Giroud JP, Willoughby DA (1971) Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J Pathol 104:15–29. https://doi.org/10.1002/PATH.1711040103
Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea J (2017) JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. Nat Rev Drug Discov 1612(16):843–862. https://doi.org/10.1038/nrd.2017.201
Shaheen N, Tukun AB, Islam S, Irfan NM, Khan IN, Hasan T (2016) Evaluation of functional potentiality of selected commonly consumed foods of Bangladesh. Funct Foods Heal Dis 6:735. https://doi.org/10.31989/ffhd.v6i11.278
Sipos F, Firneisz G, Muzes G (2016) Therapeutic aspects of c-MYC signaling in inflammatory and cancerous colonic diseases. World J Gastroenterol 22:7938–7950. https://doi.org/10.3748/wjg.v22.i35.7938
Tavakkoli A, Mahdian V, Razavi BM, Hosseinzadeh H (2017) Review on clinical trials of black seed (*Nigella sativa*) and its active constituent, thymoquinone. J Pharmacopuncture 20:179–193. https://doi.org/10.3831/KPI.2017.20.021
Tu W, Wang H, Li S, Liu Q, Sha H (2019) The anti-inflammatory and anti-oxidant mechanisms of the Keap1/Nrf2/ARE signaling pathway in chronic diseases. Aging Dis 10:637. https://doi.org/10.14336/ad.2018.0513
Whittle BA (1964) The use of changes in capillary permeability in mice to distinguish between narcotic and nonnarcotic analgesics. Br J Pharmacol Chemother 22:246–253. https://doi.org/10.1111/j.1476-5381.1964.tb02030.x
Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced edema in hind paw. Exp Biol Med 3:544–547. https://doi.org/10.3181/00379727-111-27849
Winter CA, Risley EA, Nuss GW (1962) Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med 111:544–547. https://doi.org/10.3181/00379727-111-27849

Publisher's Note