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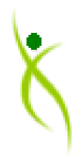

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Research Article

Pharmacology for drug discovery

Antinociceptive effect, acute toxicity and chemical analysis of cold mechanically extracted *N. Sativa* seed oil

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Abstract: *Nigella sativa* L. (Ranunculaceae), seed oil is traditionally used for pain, stiffness in the joints and eczema. The *N. sativa* seed oil (NSSO) contains an abundance of monoterpenes and essential fatty acids of various pharmacological actions. The objective of this study is to investigate the antinociceptive effect of single oral dose of NSSO on acetic acid-induced writhing and hot plate induced algesia in mice. Further, the study also, aims to characterize the chemical constituents of NSSO by using GC/MS. Swiss albino mice were divided into two sets of three groups consisting of six animals per group for the assessment of analgesic effect by hot plate and by acetic acid induced writhing methods. Group I, received distilled water (10 ml/kg, p.o.); group II, received diclofenac sodium (2.5 mg/100g, p.o.); and group III, received NSSO (0.5 ml/100g, p.o.). After NSSO administration the animals were placed on the hot plate and their response was noted down at different time interval. Further, another set of animals of other three groups were subjected to acetic acid induced writhing test after 30 minutes of drug administration. The results of the study showed that NSSO significantly ($p<0.05$) increased the latency to thermal stimulus, and also, there was significant ($p<0.01$) reduction in the number of writhes induced by 0.07% acetic acid. The GC/MS chromatogram of NSSO showed a total of 50 compounds. The LD_{50} of NSSO was greater than 5 g/kg. Thus in conclusion, NSSO (0.5ml/100g, p.o.) showed a significant analgesic effect against acetic acid induced writhing and also in hot plate model in mice. The phytochemical analysis of NSSO showed the presence of several active principles, primarily linoleic acid and thymoquinone. The presence of linoleic acid and thymoquinone in NSSO, possibly contributed to its analgesic effect that needs further investigation in other animal models of algesia.

Keywords: *N. sativa* seed oil; acute toxicity; GC-MS, analgesic and antinociceptive activity.

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1. INTRODUCTION

The seeds of *Nigella sativa* L. (Ranunculaceae), known as black seed or black cumin. *N. sativa* seed oil (NSSO), is an essential, golden-yellow to brownish-yellow colored aromatic oily liquid, volatile with a characterized strong odor, and has a lower density than that of water. The NSSO has been traditionally used for pain, stiffness in the joints, eczema[1], asthma and cough[2]. It can be obtained by different types of distillations (hydration, steam, or dry) or by cold mechanical process[3]. Several researchers subjected the NSSO to a range of pharmacological and phytochemical studies since 19th century. The NSSO demonstrated liver protection against CCl_4-induced liver toxicity in rats[4], lipopolysaccharides-induced inflammation[5], and chemical-induced carcinogenesis[6]. The NSSO also showed antinociceptive and anti-inflammatory activity in mice[7-9]. The NSSO showed inhibition of eicosanoid generation and membrane lipid peroxidation[1], and also, the antioxidant activity in both in vitro and in vivo as the most reported mechanism for its anti-inflammatory action[5,10]. The NSSO composes three main classes of constituents that include terpenes (monoterpenes & sesquiterpenes), phenylpropanoids, and different essential fatty acids[11-13]. Interestingly, the phenylpropanoids are one of the most abundant secondary metabolites in the plant, which exert protective effects against biotic and abiotic stress and modulate the inflammatory pathways in human cells. They triggered diverse pro-inflammatory cytokines in vitro and inhibited inflammation in various tissues in vivo[14]. Although most studies have linked the NSSO-induced analgesic and anti-inflammatory actions to the presence of monoterpene (thymoquinone)[15], the essential fatty acid (linoleic acid) presents in abundance in the NSSO plays a contributory role[16]. The essential fatty acid (linoleic acid) in NSSO has shown anti-inflammatory effects[17,18]. So, suggesting that NSSO may prove beneficial to the treatment of inflammatory pain. Currently, there is a high demand for a potent analgesic agent with new mechanism/s of action and fewer side effects, as a significant number of people around the world use analgesics for resolving a variety of pain disorders. The objective of this study is to investigate the antinociceptive effect of NSSO and its chemical profile using writhing and hot plate nociceptive models in mice and GC/MS analysis respectively. To the best of the authors' knowledge, no study has investigated the analgesic effects of whole *N. sativa* seed oil along with its chemical characterization. Majority of the studies focused mainly on different fractions or one or more active principles for investigating their analgesic effect. It is therefore of great to investigate the analgesic effect of NSSO following toxicity testing and chemical characterization, to provide supporting scientific evidence for its potential benefit in the management of pain in traditional medicine.

2. MATERIALS AND METHODS

2.1 *Drugs and chemicals*

N. sativa seed oil (Ranunculaceae) obtained from RCO GROUP SDN BHD, Shah Alam, 41300, Selangor, Malaysia. Diclofenac sodium (Novartis GSK, India) and acetic acid solution (GLOBAL Chemie, USA). The other chemicals and solvents used were analytical grade.

2.2 *Animals*

Handling and care of animals were conducted according to the European guidelines (Directive 2010/63/EU) on the protection of animals used for scientific purposes in agreement with the NIH guidelines. This study was carried out in strict compliance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health[19]. The Research and animal ethical Committee approved the protocol at the Unaizah College of Pharmacy, Qassim University (Permit Number: 22/01/2018). Female Sprague-Dawley rats (170-200 g) and albino mice (25-30 g) obtained from animal house, Qassim University. The experimental animals were housed in propylene cages (6 animals/cage) in an air-conditioned room maintained at 22 ± 2°C and 55% ± 15% humidity with a 12 h light and 12 h dark cycle. The animals were feed on standard animal food and water *ad libitum*. The animals were fasted for 10 hr before the start of the experiment. The NSSO (0.5 ml/100g) and diclofenac sodium (2.5 mg/100g, p.o.) were administrated orally.

2.3 *Antinociceptive activity*

2.3.1 *Hot plate method in mice*

The hot plate test was performed to evaluate the central antinociceptive action of the NSSO using a hot-plate apparatus (Harvard, UK) as described previously[20-21]. The temperature of the apparatus was set at 55 ± 1.0 °C. Mice averagely weighing 24.5 ± 4.2 g (n= 6 mice/group) that showed nociceptive responses within 15 s were distributed randomly into three groups. The overnight fasted mice of group I, II and III received distilled water (0.5 ml/100g, p.o.), NSSO (0.5 ml/100 g, p.o.)[5,22] and diclofenac (2.5 mg/100 g, p.o.) as standard drug respectively. Each mouse was placed on the hot-plate apparatus, and the latency of mice was recorded before (baseline), at 30, 60, and 120 min following treatment. The latency to thermal-induced nociceptive pain was recorded when the animal first licked its paws, lifting off its paws or started to jump. A cut-off nociceptive response of 60s was set to avoid mouse tissue damage. Results obtained were expressed as mean hot plate response latency ± SEM(s).

2.3.2 *Acetic acid induced writhing in mice*

The acetic acid-induced writhing nociceptive was carried out to investigate the peripheral analgesic action of NSSO, as described previously by Siegmund et al. and Collier et al [23,24]. Mice averagely weighing 24.5 ± 4.2 g (6 mice/group) were assigned randomly into three groups. The overnight fasted mice were pre-treated with 0.5 ml/100 g of NSSO or diclofenac (2.5 mg/100g) or distilled water (DW) 0.5ml/100g. The NSSO, diclofenac, and DW groups were given p.o, 30 min before induction of writhing by 0.7%, 0.1 ml/100g acetic acids (i.p.). Writhing nociceptive response was quantified after 5 min of latency for each animal over 30 min. The observation of the writhing response includes an extension of the trunk, hind limbs, and abdominal muscles. The percentage of analgesic activity against acetic acid-induced writhing was calculated as follows:

% Analgesic activity = (Mean No. of writhes (Control) - Mean No of writhes (Treated))/(Mean No. of writhes (Control)) ×100

2.3.3 *Acute toxicity studies (LD_{50} determination)*

Acute toxicity test was carried out on ten female Sprague-Dawley rats according to OECD guidelines 425 revised up-and-down procedure for testing of chemicals[25]. Healthy, virgin female Sprague-Dawley rats (age of 7-10 weeks) weighing 150–180 g were used. The animals were randomly divided into two groups (n = 5). On day one, overnight fasted female Sprague-Dawley rat was given a single oral dose of 5.2 mL/kg of NSSO, which is equivalent to 5g/kg. The rat was continuously monitored for 24 h for possible behavior or physical changes. Special attention was given to the first 4 hrs. After 24h, four remaining rats were given the same dose and monitored daily for 14 days for toxicity manifestations (tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma). Another group without treatment was used as control group. After 14 days, all the animals were sacrificed by euthanasia in a CO_2 chamber, and vital organs (heart, liver, kidney, lung, adrenal gland, spleen, uterus, ovaries, thymus, brain and an empty stomach) were excised, weighed, and microscopically examined.

2.3.4 *Gas Chromatography-Mass spectrometry (GC-MS) analysis of NSSO*

An Agilent 6890N/5973N CI/EI series GC/MS system (Agilent Technologies, USA) was used for the chromatographic analysis. An Agilent system comprising an auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Agilent 19091S-433 HP-5MS (5% Phenyl Methyl Siloxane) fused a capillary column (30 m length × 250 μm diameter × 0.25 μm film thickness). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas was used as a carrier gas at a constant flow rate of 1.2 ml/min, and an injection volume of 1 μl was employed (a split ratio of 15:1). The initial oven temperature at 70°C and maximum temperature was 325°C. The front inlet temperature was 280°C with pressure at 10.94 psi. The total run time was 32.50 min. Mass spectra were taken at 70 eV. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The software adapted to handle mass spectra and chromatograms was ChemStation software. Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), having more than 107886 compounds. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained [26-27].

3. STATISTICAL ANALYSIS

All the data were represented as mean ± SEM and subjected to statistical analysis using SPSS® ver. 23 Software. The obtained data were analyzed by one-way analysis of variance (ANOVA) followed by Post Hoc Dennett's test. The level of significant difference was determined at $p < 0.05$, as described by Duncan-Johnson, and Donchin.

4. RESULTS

4.1 *Antinociceptive effect of NSSO on hot plate model*

As shown in graph 1a, NSSO increased the response latency to thermal stimulus when compared with the control group. Treatment with NSSO 0.5 ml/100g showed significant ($p < 0.01$) antinociceptive activity at the time of 30 min to 120 min after the treatment, increasing the latency time when compared with the control. The positive control group, diclofenac (2.5 mg/100g), increased the response latency to heat stimulation 30 min after treatment.

4.2 *Antinociceptive effect of NSSO on acetic acid-induced writhing model*

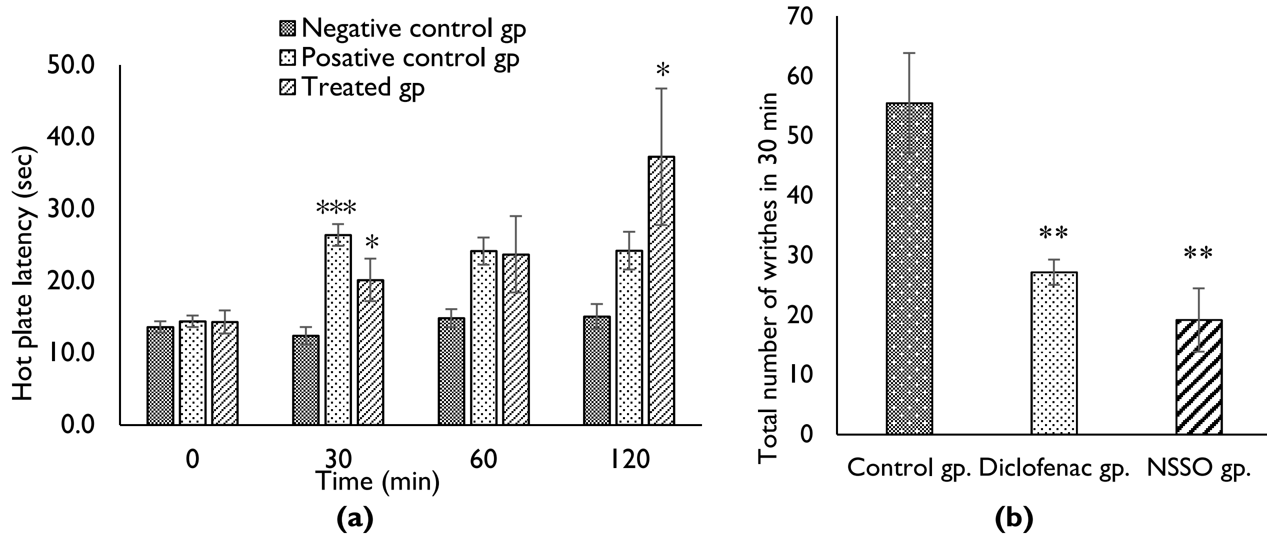
As shown in graph 1b, mice develops abdominal writhes as a result of painful stimuli induced by the injection of 0.7% acetic acid (1 mL/100 g, i.p.). Mice pre-treated (30 min) with the diclofenac 2.5 mg/100g or NSSO (0.5 ml/100g, p.o.) showed a significant ($p < 0.01$) reduction in the mean number of writhes (27.17 ± 2.12; 19.17 ± 5.31) when observed over 30 min compared to the control group (55.50 ± 8.38).

4.3 *Acute toxicity studies (LD_{50} determination) of NSSO*

The measured dose of 5.2 ml/kg of the NSSO is devoid of any toxicity and did not cause any significant behavioral changes such as drowsiness, salivation, tremor, restlessness, convulsion, piloerection, diarrhea in the treated rats in the first 24 h observation period and also during the two-week follow-up period. Moreover, there was no statistically significant difference in their body weight and organ-to-body weight ratios observed as compared to the control group (Table 1).

4.4 *Phytochemical analysis of NSSO by GC/MS*

GC/MS analysis of NSSO based on a comparison of retention indexes and mass spectra allowed the identification of chemical compounds between 3 and 30 minutes. The chromatogram is shown in figure 1, and compounds identified in the essential oil are given in table 2. A total of 50 compounds were identified by matching their spectra with the NIST14 Library and literature. The compounds with their retention time (RT), molecular formula, molecular weight (MW), and peak area (%) are presented in table 2 as reported previously[28]. Among the 50 identified compounds, 12 were more than 1% concentration. The most abundant components were 9,12-Octadecadienoic acid (Z,Z) (21.03%), 2,5-Cyclohexadiene-1,4-dione, 2-methyl-5-(1-methylethyl) (15.25%), Benzene, 1-methyl-2-(1-methylethyl) (14.6%), Bicyclo[3.1.0]hex-2-ene, 2-methyl- 5-(1-methylethyl) (5.58%), 2',6'-Dihydroxy-3'-methylacetophenone (3.96%), n-Hexadecanoic acid (3.56%), 3-Methyl-4-isopropylphenol (2.83%), and 1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.a lpha., 3a.beta., 4.alpha., 8a.beta.)] (2.26%).



(a) **(b)**

*Values were expressed as mean ± SEM (n = 6). ***p<0.001, **p<0.01, *p<0.05 when compared with respective control, significant by ANOVA followed by Dunnett's t-test. (a) Using heat-induced analgesia; (b) using the number of writhes acetic acid-induced within 30 minutes in mice.*

Graph 1: Analgesic effect of *Nigella sativa* seed oil (NSSO) in mice using hot plate and acetic acid induced writhing methods

Table 1. Organ-to-body weight ratios of rats treated with NSSO (5.2 ml/kg, p.o.) for acute toxicity study

Organs	*NSSO* (5.2 mL/kg) (Mean ± SEM)	Control, water (5mL/kg) (Mean ± SEM)
Heart	0.34 ± 0.02	0.35 ± 0.01
Liver	3.12 ± 0.10	3.12 ± 0.09
Kidney (R)	0.32 ± 0.01	0.32 ± 0.01
Kidney (L)	0.32 ± 0.01	0.32 ± 0.01
Lung	0.59 ± 0.01	0.63 ± 0.04
Adrenal (R)	0.02 ± 0.00	0.02 ± 0.00
Adrenal (L)	0.02 ± 0.00	0.02 ± 0.00
Spleen	0.23 ± 0.01	0.26 ± 0.01
Uterus + ovaries	0.42 ± 0.02	0.45 ± 0.03
Thymus	0.20 ± 0.01	0.19 ± 0.01
Brain	0.91 ± 0.03	0.85 ± 0.03
Empty stomach	0.63 ± 0.02	0.67 ± 0.03

Values were expressed as mean ± SEM, n=5. P>0.05, insignificant when compared with control by one way ANOVA.

Table 2. Chemical Constituents identified in NSSO by GC-MS.

No.	RT	Compounds	MF	MW	PA%
1	3.823	Bicyclo[3.1.0]hex-2-ene, 2-methyl- 5-(1-methylethyl)-	$C_{10}H_{16}$	136.24	5.58
2	3.914	α-Pinene	$C_{10}H_{16}$	136.24	1.45
3	4.351	β-Pinene	$C_{10}H_{16}$	136.24	1.98
4	4.719	Bicyclo[4.1.0]hept-2-ene, 3,7,7-trimethyl	$C_{10}H_{16}$	136.24	0.36
5	4.809	Benzene, 1-methyl-2-(1-methylethyl)-	$C_{10}H_{14}$	134.22	14.6
6	5.108	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	$C_{10}H_{16}$	136.24	0.26
7	5.934	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1R)	$C_{10}H_{16}O$	152.24	0.01
8	6.177	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)	$C_{10}H_{18}O$	154.25	0.42
9	6.801	Thymoquinone (2,5-Cyclohexadiene-1,4-dione, 2-methyl-5-(1-methylethyl)-	$C_{10}H_{12}O_2$	164.204	15.25
10	6.926	Benzaldehyde, 2-hydroxy-5-methoxy-	$C_8H_8O_3$	152.149	0.16
11	7.093	3-Methyl-4-isopropylphenol	$C_{10}H_{14}O$	150.22	2.83
12	7.572	Tricyclo[5.4.0.0(2,8)]undec-9-ene,2,6,6,9-tetramethyl	$C_{15}H_{24}$	204.36	0.45
13	7.996	Longifolene (1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1.alpha.,3a.beta.,4.alpha.,8a.beta.)])	$C_{15}H_{24}$	204.36	2.26
14	8.322	Phenol, 3-(1,1-dimethylethyl)-4-methoxy	$C_{11}H_{16}O_2$	180.25	0.08
15	8.537	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-,(S)	$C_{15}H_{24}$	204.36	0.09

16	8.704	Isoledene	$C_{15}H_{24}$	204.36	0.10
17	8.787	2',6'-Dihydroxy-3'-methylacetophenone	$C_9H_{10}O_3$	166.17	3.96
18	9.016	7-Tetradecenal, (Z)-	$C_{14}H_{26}O$	210.36	0.17
19	9.113	Tetradecanal	$C_{14}H_{28}O$	212.38	0.09
20	9.384	Cyclododecene, (E)-	$C_{12}H_{22}$	166.31	0.04
21	9.474	1-Tetradecene	$C_{14}H_{28}$	196.38	0.05
22	9.689	1H-Cycloprop[e]azulene,1a,2,3,4,4a,5, 6,7b-octahydro-1,1,4,7-tetramethyl-, [1aR-(1a.alpha.,4.alpha.,4a.beta.,7b. alpha.)]	$C_{15}H_{24}$	204.36	0.12
23	9.988	Cyclododecene	$C_{12}H_{22}$	166.31	0.13
24	10.078	Butyric acid, tetradecyl ester	$C_{18}H_{36}O_2$	284.48	0.13
25	10.807	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.46	0.21
26	11.029	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.43	3.56
27	11.279	Nerolidol 1	$C_{15}H_{26}O$	222.37	0.38
28	11.661	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.48	0.81
29	11.960	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	21.03
30	12.216	13-Tetradece-11-yn-1-ol	$C_{14}H_{24}O$	208.35	1.66
31	12.494	1,3,3-Trimethyl-2-hydroxymethyl-3,3-dimethyl-4-(3-methylbut-2-enyl) cyclohexene	$C_{15}H_{26}O$	222.37	0.51
32	12.876	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294.48	0.23
33	13.043	2-Chloroethyl linoleate	$C_{20}H_{35}ClO_2$	342.95	0.10
34	13.140	3-Eicosene, (E)-	$C_{20}H_{40}$	280.54	0.33
35	13.501	9,12-Octadecadienal	$C_{18}H_{32}O$	264.45	0.17
36	13.605	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	$C_{19}H_{38}O_4$	330.51	0.34
37	13.820	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	0.07
38	14.778	Ethanol, 2-(9,12-octadecadien yloxy)-, (Z,Z)	$C_{20}H_{38}O_2$	310.51	1.56
39	15.861	Squalene	$C_{30}H_{50}$	410.73	0.10
40	17.104	Oxirane, 2,2-dimethyl-3-(3,7,12,16, 20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-, (all-E)	$C_{30}H_{50}O$	426.73	0.11
41	18.020	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all E)	$C_{30}H_{50}$	410.73	0.16
42	18.694	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraphenyl)-cyclohexanol	$C_{30}H_{52}O$	428.75	0.04
43	19.013	Eicosane	$C_{20}H_{42}$	282.56	0.03
44	19.874	Vitamin E	$C_{29}H_{50}O_2$	430.72	0.10
45	21.790	Campesterol	$C_{28}H_{48}O$	400.69	0.11
46	22.463	Stigmasterol	$C_{29}H_{48}O$	412.70	0.29
47	23.796	Stigmasterol, 22,23-dihydro-	$C_{29}H_{50}O$	414.72	0.64
48	25.691	Silane, (9,19-cyclo-9.beta.-lanost-24-en-3.beta.-yloxy)trimethyl	$C_{33}H_{58}OSi$	498.91	0.14
49	27.524	Thieno[2,3-b]pyridine-2-carbonitrile, 3-amino-4-dimethylamino	$C_{10}H_{10}N_4S$	218.28	0.12
50	28.961	9,19-Cyclolanost-24-en-3-ol, acetate	$C_{32}H_{52}O_3$	484.77	0.28

a Compounds listed in order of elution from 19091S-433 HP-5ms polyimide coated capillary column (30 m × 0.25 mm, 0.1 μm; Agilent Technologies). bCompounds were identified based on computer matching of the mass peaks with National Institute Standard and Technology (NIST02) Library MF= Molecular Formula; MW= Molecular Weight; PA%= Peak Area%; RT= Retention Time

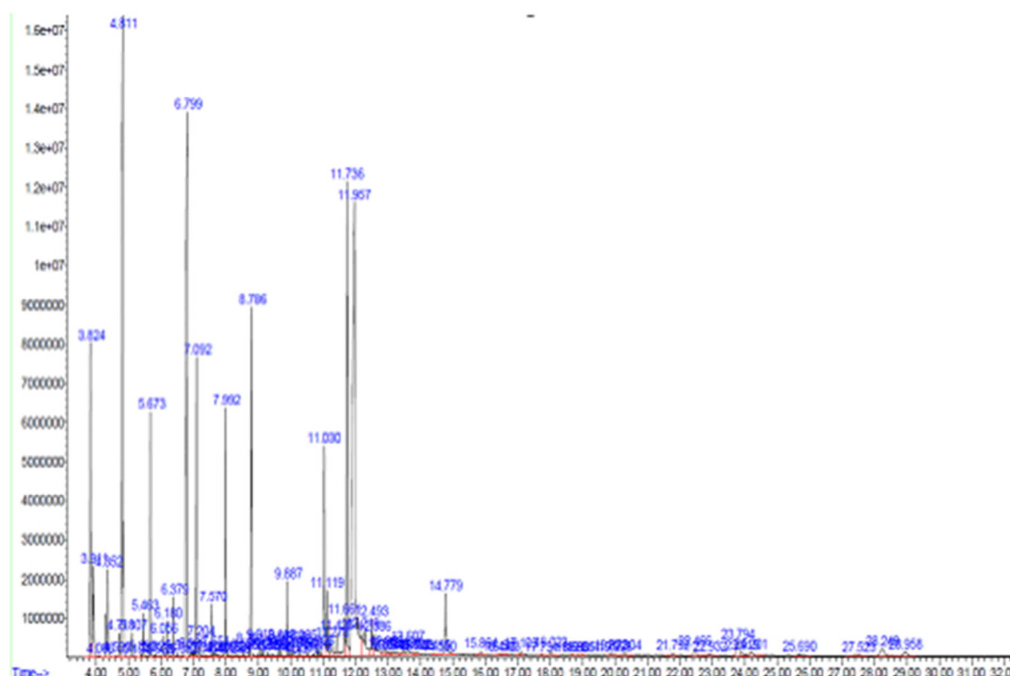


Fig 1. GC-MS chromatogram obtained by GC/MS analysis of NSSO

5. DISCUSSION

NSSO is an essential oil with well-acknowledged benefits traditionally in the relief of pain, stiffness in the joints, and eczema[1,2]. The objective of the study is to investigate the antinociceptive effect of NSSO using writhe and hot plate nociceptive models in mice and its chemical profile using GC/MS. In this study, the NSSO showed antinociceptive effect. Thymoquinone, as well as linoleic acid, were the principal constituents in the phytochemical profile using gas chromatography-mass spectrometry (GC-MS). The hot plate nociception model is a thermic stimulus involving stimulation of the pain pathway and controlled by the central nervous system. So, the thermal stimulus was used to evaluate the anti-nociceptive activity mediated by central mechanisms[29]. In this model of nociception, two behavioral responses, namely paw licking and jumping, are induced. The two responses are supraspinal integrated and sensitive to centrally acting analgesics, such as opioid derivatives[30]. The oral acute single dose of NSSO has shown a significant effect at all-time points ($p<0.05$) when compared with control. We demonstrated that NSSO exerts significant prolongation in the latency response time against thermal stimuli. Interestingly, our results are in line with the previous study that reported the implication of central mechanism in the anti-nociceptive effect of NSSO, wherein it has been found that naloxone significantly blocked the *N. Sativa* oil and thymoquinone-induced antinociception in the early phase of the formalin test.[9] The central antinociceptive effect of NSSO directly linked to its monoterpenoid content, such as α-pinene[31,32] and β-pinene[33]. β-pinene was reported to have supraspinal antinociceptive action[34]. However, it appears that at least a part of the analgesic effects of NSSO is due to the presence of thymoquinone, although it is not so clear whether this compound is the only contributing component of this essential plant oil or not. The acetic acid-induced nociception model involves the non-selective cationic channels in the peripheral nociceptive neurons (C fiber), causing the release of pain mediators such as prostaglandins (PGs), cytokines (IL-8, TNF-α, and IL-1β), cyclooxygenase (COX), lipoxygenase (LOX), serotonin, bradykinin, and histamine in the peripheral tissue fluid[33]. Therefore, acetic acid (AA)-induced writhing was used as a stimulus to evaluate the anti-nociceptive activity mediated by the peripheral nervous system[35]. In this model of nociception, behavioral responses, namely abdominal constrictions, an extension of the forelimbs, and elongation of the body [36,37] are evaluated. These responses are dorsal horn integrated, and sensitive to peripherally acting analgesics such as NSAID derivatives[38] because increased levels of the pain mediators provoke peripheral nociceptive neurons entering into the dorsal horn of the central nervous system[38]. The oral acute single dose of NSSO showed a significant effect ($p < 0.01$) in AA-induced (nociception model) behavioral responses when compared with control. We demonstrated that NSSO exerted significant prolongation in the latency response time to AA stimuli and reduced the nociceptive behavioral responses. These results suggest that the anti-nociceptive effect of NSSO shown in the AA model could be driven from anti-nociceptive action involving peripheral mechanisms. The antinociceptive effect of NSSO might be attributed to the blockade of the release of endogenous inflammatory mediators or direct inhibitory activity at nerve endings of the primary afferent neurons and/or inhibition of the transmission pathway entering the dorsal horn. In addition to this, thymoquinone has been shown to downregulate the tumor necrosis factor[39] and suppress of the nuclear factor kappa B (NF-kB) activation in the brain and spinal cord[40]. El-Mahmoudy et al [40] reported that thymoquinone significantly suppressed the expression of inducible nitric oxide synthase (iNOS) in rat macrophages[41]. Moreover, the peripheral anti-nociceptive effect of NSSO is directly linked to its α-Pinene, which has an anti-inflammatory effect via PGE-1[42], and may control nociception at dorsal horn through GABA receptors[43]. The GABA inhibitory neurons were found abundance in the spinal cord and have strongly been suggested its to have essential functional roles in pain inhibition[44]. Further, 4-Terpineol suppress pro-inflammatory mediator production in vitro of TNF-α, IL-1, IL-8, IL-10, and PGE2 by LPS-activated human blood monocytes[45,46]. 4-Terpineol also showed antioxidant activity[17]. Thymoquinone, which showed an anti-nociceptive effect on different

nociceptive models, was shown to have central and peripheral effects[8,47]. Oil with Thymol as abundant constituent showed anti-inflammatory effect through lowering of the amounts of IL-1β and IL-6 cytokines in mice with colitis induced by trinitrobenzene sulphonic acid (TNBS)[48]. The oil with a high concentration of thymol was reported to significantly inhibit total mRNA IL-1β expression in mice with TNBS-induced colitis[49]. Moreover, antioxidant activities of the essential oils are well documented,[17] including the antioxidant inhibitory effect of NSSO volatile constituents on inflammatory procedure.[5] The phytochemical profile of NSSO was comprehensively characterized using gas chromatography-mass spectrometry (GC-MS). A total of 50 different compounds were identified. Among them, six compounds, namely α-pinene[51], β-pinene[34], vitamin E[51], thymoquinone[52], stigmasterol[53] and linoleic acid[17,18] have been reported to possess antinociceptive activity (see Table 2 & Fig.1).

6. CONCLUSION

The result of the acute toxicity study clearly showed that oral administration of NSSO was safe at the evaluated dose, and the LD_{50} is more than 5.2 mL/kg. Furthermore, the monoterpene and fatty acid-rich *N. sativa* seed oil showed anti-nociceptive and anti-inflammatory effects by modifying pain neuronal cascades and scavenging of free radicals from the site of injury and inhibiting the synthesis of prostaglandins and leukotrienes. Thymoquinone, α-thujene, α-pinene, β-pinene, 4-terpineol, thymol as monoterpene class, and vitamin E, and stigmasterol as steroid derivatives of NSSO constituents may have a role in its antinociceptive effects. Fatty acids present in abundant amount in NSSO, such as linoleic acid, represents more than 5% of the total constituents and it would be interesting to assess its antioxidant and anti-inflammatory activity in an intact cell.

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8. AUTHORS CONTRIBUTION STATEMENT

Mahfoudh A. M. Abdulghani and Redhwan Ahmed Al-Naggar conceived and presented research idea. Mahfoudh A. M. Abdulghani and Mohammed Ali Ahmed Saeed supervised the finding of this work. Mohammad Razak Hamdan carried out the chemical analysis. Mahfoudh A. M. Abdulghani and Md Jamir Anwar drafted the manuscript. Bala Yauri Muhammad contributed to the interpretation of the results. All authors discussed the results and contributed to the final manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

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