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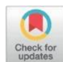

Original Article

In vivo Acute Toxicity, Analgesic Activity and Phytochemical Characterization of *Solenostemma argel* (Del) Hayne Essential Oil

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Abstract

Introduction: *Solenostemma argel* is a Saharan plant used in traditional medicine to cure pain. The present work investigated the quantitative analysis of the composition of the essential oil of *S. argel* leaves (EOSA) as well as its acute toxicity and anti-nociceptive activity.

Materials and Methods: The chemical characterization of EOSA was carried out by GC-MS and NMR. EOSA acute oral toxicity study was performed according to the OECD-420 method. EOSA anti-nociceptive activities were evaluated by acetic acid-induced abdominal writhing test, hot plate test, and formalin test.

Results: Twenty components were identified by GC-MS including Linanool (57.10%), terpineol (12.95%), trans-geraniol (12.65%), and nerol (4.67%). The main compound linalool was isolated by NMR. The EOSA at 250 and 400 mg/kg significantly attenuated acetic acid-induced writhing by 72.71 and 92.41%, respectively. Moreover, Ingestion of EOSA at doses of 250 and 400 mg/kg caused a significant and dose-dependent anti-nociceptive effect in both neurogenic and inflammatory phases of formalin-induced licking. EOSA impacts the pain latency in the hot plate test.

Conclusions: The results of this study showed that EOSA has an anti-nociceptive effect on central and peripheral pain.

Keywords: GC-MS, NMR, Acute Toxicity, Analgesic, *Solenostemma argel*

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Introduction

Pain is one of the most widespread and important health problems due to its prevalence and the disability it can induce. The main role of pain is the protection of the organism against stimuli capable of damaging tissues through the activation of reflex mechanisms of spinal withdrawal.[1] Indeed, acute pain serves as an alarm and has the merit of protecting the body against harmful stimuli, while chronic pain can be a harbinger of inflammatory diseases or tissue damage such as nerve damage in the case of neuropathic pain.[2]

Analgesic drugs are amply used for the treatment of pain. However, their overuse leads to side effects particularly liver and kidney damage, gastrointestinal disorders, addiction, and respiratory discomfort. Recently, many natural products from medicinal plants were shown as effective and safe for the treatment of various diseases with pain.[3] In the search for new active molecules against pain, essential oils can represent an interesting source thanks to their richness in active components.

S. argel is a Saharan plant growing in the desert zones of Algeria, Libya, and Egypt.[4,5] In southern Algeria it is one of the most used plants in traditional medicine by the Hoggar people to treat various symptoms including pain.[6] The aerial parts of *S. argel* have been used traditionally in different preparations for the treatment of pain related to stomach ache, kidney pain,[7] neuralgia, sciatica,[8] and rheumatism.[5] Effectively, in our precedent study, we proved the effectiveness of acetone extract from the leaves of *S. argel* on peripheral and central pain.[6] Another study carried out by Mudawi and collaborators also demonstrated the analgesic

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effect of the aqueous extract of *S. argel* on a mouse model.[9]

This study was undertaken with the aim of highlighting for the first time the analgesic effect of the essential oil of *S. argel* (EOSA).

Materials and Methods

Chemicals and Drugs

Acetic acid, formalin, the standard drugs indomethacin, and morphine sulphate were purchased from Sigma Aldrich Corporation (St. Louis, MO, USA).

Plant

S. argel was collected in January 2021 at Abalessa, Tamanrasset (22° 47′ 13″ North, 5° 31′ 38″ East) - Algeria. The plants were dried at room temperature, in a dry and aerated place. The identification of the studied plant was carried out by the botanical department of the "Ecole Nationale Supérieure d'Agronomie" (ENSA) in Algiers, Algeria and they delivered us a certificate of identification. Voucher specimens were deposited in the herbarium of "Santé et Productions Animales" Laboratory of the "Ecole Nationale Supérieure Vétérinaire" of Algiers (SPA.031).

Extraction of the Essential Oil

Extraction of the essential oil of *S. argel* was performed by hydrodistillation using a Clevenger-type apparatus. One hundred and fifty grams of crushed leaves are put in a 1000 ml round flask and then distilled water added to the leaves. The mixture is hydrodistilled for 4 h. The resulting oil was stored in an amber bottle at 4 °C until use. The percentages of EOSA were calculated as volume (ml) of EOSA per 100 g of *S. argel* leaves (v/w).

GC-MS Analysis

EOSA was analysed by GC-MS. The GC-MS analysis was carried out using an Agilent Technologies 7890A series gas chromatography interface with Agilent 5975 C mass selective detector. Instrument and data acquisition performed with Chem-station software (Agilent Technologies, Wilmington, DE, USA).

The analytical capillary column was HP5-MS (30 m × 0.25 mm i.d, 0.25 mm film thickness). The injector temperature was 250 °C and injection volume was 0.2 µl and the split ratio 1:50. The over-temperature program was 60 °C for 8 min, 2 °C/min to 250 °C for 20 min. The temperature of the MS source and quadrupole source was respectively 230 °C and 150 °C, impact of the ionization mode was 70 eV and over a scan range of 29-550.

Identification of compounds was carried out based on the GC retention indices calculated from a series of alkanes injected under the same conditions with the sample, and by comparing the mass spectra with those of the Wiley 7 NIST 2006 library. The percentage of EOSA compounds was calculated from the GC peak areas.

NMR Analysis

NMR spectra were recorded on a Bruker Avance 300 spectrometer (Bruker Daltonics, Bremen, Germany) (300.13 MHz for ^1H and 75.47 MHz for ^{13}C). Tetramethylsilane (TMS) was used as the internal standard. The chemical shifts (δ, ppm) described for each compound were obtained at room temperature in a solution of deuterated chloroform ($CDCl_3$).

Unequivocal ^{13}C NMR assignments were made with the aid of 2D gHSQC and gHMBC (delays for one-bond and long-range J C/H couplings were optimised for 145 and 7 Hz, respectively) experiments.

Animals

Healthy Wistar albino rats (150-200 g) and Swiss albino mice (20-30 g) of both sexes (Institute Pastor of Algiers, Algeria). All animals were housed in an animal room under standard laboratory conditions of temperature (25 ± 2 °C), relative humidity (55 ± 1%), 12 h light/dark cycle, and fed with standard pelleted food and water ad libitum. The study was permitted by the scientific council of "Santé et Productions Animales" Research Laboratory of the Higher Veterinary School of Algiers, in accordance with the ARRIVE guidelines 2.0.[10]

Acute Toxicity Study

An acute oral toxicity study was performed following the guidelines of the Organization for Economic Co-operation and Development.[11] Nulliparous and non-pregnant healthy female rats were used for this study. The rats were divided into three groups with five animals in each group. A single dose of the essential oil of *S. argel* (EOSA) (500, 1000, and 2000 mg/kg) was administered to overnight fasted rats, while the control group received vegetable oil (10 ml/kg). Animals were observed individually during the entire experimental period, for any behavioural and neurological changes as a sign of acute toxicity. For 14 days, the animals were weighed, and the number of deaths was considered. On the 14th day, blood samples are taken to analyse some biochemical parameters including urea, creatinine, AST, ALT, alkaline phosphatase, total protein, and albumin. Then, the rats were sacrificed, dissected and different organs (hearts, livers, kidneys, lungs, and spleens) were histologically analysed.

Analgesic Activity

Writhing Test in Mice

The assay was performed according to the previously described.[6,12] Briefly, the nociceptive effect caused by the injection of acetic acid was detected by observing abdominal writhing associated with stretching of the whole body. Four

groups of 6 mice each were treated with EOSA (250 and 400 mg/kg, p.o.), indomethacin (10 mg/kg p.o.), and vegetable oil (10 ml/kg, p.o.). The significant reduction in writhes the number of treated groups was compared to that of the control and standard groups.

Formalin Test

The formalin test was carried out as previously described.[6,13] Briefly, four groups of 6 rats, each one was treated orally with vegetable oil (10 ml/kg), Morphine (10 mg/kg), and EOSA (250-400 mg/kg). One hour after treatment, rats were injected with 50 µl of 2.5% formalin (in 0.9% saline) in the ventral surface of the right hind paw and the duration of paw licking was determined 0-5 min (first phase), and 20-25 min (second phase) after formalin injection. A nociceptive score was determined for each phase by measuring the amount of time spent biting/licking of the injected paw.

Hot Plate Test

The test was performed as previously described.[6] Four groups of 6 mice each were treated with EOSA (250 and 400 mg/kg, p.o.), morphine (10 mg/kg p.o.), and vegetable oil (10 ml/kg, p.o.). Briefly, the time (seconds) for paw licking or jumping was taken as reaction time and was measured in regular time intervals and the reaction strength of each rat was determined before and after drug treatment at 30 min, 60 min, and 120 min. The groups administered with tested extract were compared to control and standard drug groups.[14]

Statistical Analysis

The statistical treatment of the data was carried out on the XLSTAT Version 7.1 software and the IBM SPSS Statistics Version 20 software. All values were expressed as means ± standard deviation. The results obtained were statistically analyzed by a one-factor and two-factor ANOVA followed by a post hoc multiple comparison tests of the differences between the groups by applying the Tukey HSD test. Results were considered statistically significant at $p<0.05$.

Results

GC-MS Analysis

The composition of the EOSA was performed using the GC-MS method. Table 1 illustrated the more important data of the compounds (Calculated IR, identification of compounds, theoretical IR, and %GC-MS).

Table 1. GC-MS Data from the Essential Oil of *S. argel*

N°	IR[a]	Compounds	IR[b]	%GC-MS [c]	Identification
1	983	cis-2,6-Dimethyl-2,6-octadiene		0.126	RI, MS
2	1000	trans-2-(2-Pentenyl)furan	1007	0.326	RI, MS
3	1024	D-Limonene	1025	0.903	RI, MS
4	1035	Z-β-Ocimene	1038	0.552	RI, MS
5	1045	E-β-Ocimene	1048	0.949	RI, MS
6	1083	α-Terpinolen	1085	0.885	RI, MS
7	1106	Linalool	1103	57.103	RI, MS
8	1107	1,5,7-Octatrien-3-ol, 3,7-dimethyl-	1104	1.047	RI, MS
9	1173	Terpinene-4-ol	1174	0.245	RI, MS
10	1190	Terpineol	1189	12.954	RI, MS
11	1212	p-Menth-1-en-9-al	1232	0.430	RI, MS
12	1228	Nerol (cis-Geraniol)	1226	4.678	RI, MS
13	1257	trans-Geraniol	1258	12.658	RI, MS
14	1286	Edulan I, dihydro-	1289	0.679	RI, MS
15	1379	β-Damascenone	1382	0.487	RI, MS
16	1449	Geranyl acetone	1452	0.124	RI, MS
17	1480	β-Ionene	1483	0.184	RI, MS
18	1713	Pentadecanal	1713	0.441	RI, MS
19	1844	2-Pentadecanone, 6,10,14-trimethyl-	1843	0.21	RI, MS
20	1970	n-Hexadecanoic acid	1970	0.462	RI, MS
%Identification				**95.443**	

[a]Retention indices with respect to C5-C28 n-alkanes calculated on non-polar HP5-MS capillary column; [b]Retention indices given in literature (NIST or Wiley on non-polar HP-MS or DB5-MS capillary column); [c]Percentage calculated from the peaks areas of GC chromatogram on non-polar HP5-MS capillary column.

The extraction of the essential oil from the leaves of *S. argel* by hydrodistillation yielded a very fragrant yellow oil. The yield obtained per 100 g of the plant is 0.3% (v/w). Twenty components were identified, representing 95.44% of the total EOSA components characterized.

NMR Analysis

The main component of EOSA revealed by GC-MS analysis was linalool. To confirm that, the essential oil was analysed by NMR spectroscopy. The ^1H NMR clearly shows the three methyl groups and the vinylic protons at the expected chemical shifts (Figure 1).

In the vinylic system composed of carbons C-1 and C-2, it is obvious that the two protons linked to carbon C-1 are not equivalent and appear at δ 5.05 and 5.23 ppm with coupling constants typical of geminated protons (J = ~1 Hz) and a vicinal coupling constant typical of a *cis* (J = ~11 Hz) and *trans* (J = ~15 Hz) configuration. These last constants are a

result of the coupling with proton H-2, which appears as a double doublet at δ 5.91 ppm and allows the identification of the proton H-1_{cis} δ 5.05 ppm and H-1_{trans} 5.23 ppm (Figure 1).

These identifications could be confirmed by the two-dimensional NMR experiments. The other protons could also be detected by these experiments, which also allowed the confirmation of proton H-6 assignment and the methyl group identification. The ^{13}C NMR spectrum also confirms the linalool structure and using both the HSQC and HMBC spectra was possible to identify the most relevant signs, from which carbon C-3 at δ 74 ppm can be highlighted.

Acute Toxicity

The results of the EOSA acute toxicity study are shown in Tables 2 and 3. Oral administration of EOSA at doses of 500, 1000, and 2000 mg/kg did not cause any visible symptoms of toxicity in the treated rats. No neurological toxicity or behavioural changes were observed and a progressive body weight gain was noted throughout the experimental period.

Furthermore, no significant differences were revealed regarding the levels of biochemical parameters of blood serum, except for AST (p<0.002) and ALT (p<0.002) values recorded at a dose of 2000 mg/kg compared to the control.

At the end of the test, control rats and those treated with EOSA at different doses were autopsied and their liver, kidneys, lungs, spleen, and heart were histopathological examined. The post-mortem examination revealed that the size, colour, and shape of the various organs removed in situ were normal, and no lesions of ischemic necrosis or a focus suspected of tissue damage were observed.

Histopathological examination from the control rats revealed no abnormality of the lesions. However, the hepatic lesions detected in rats treated with EOSA appeared at a dose of 2000 mg/kg and are described by light and focal venous stasis (Figure 2).

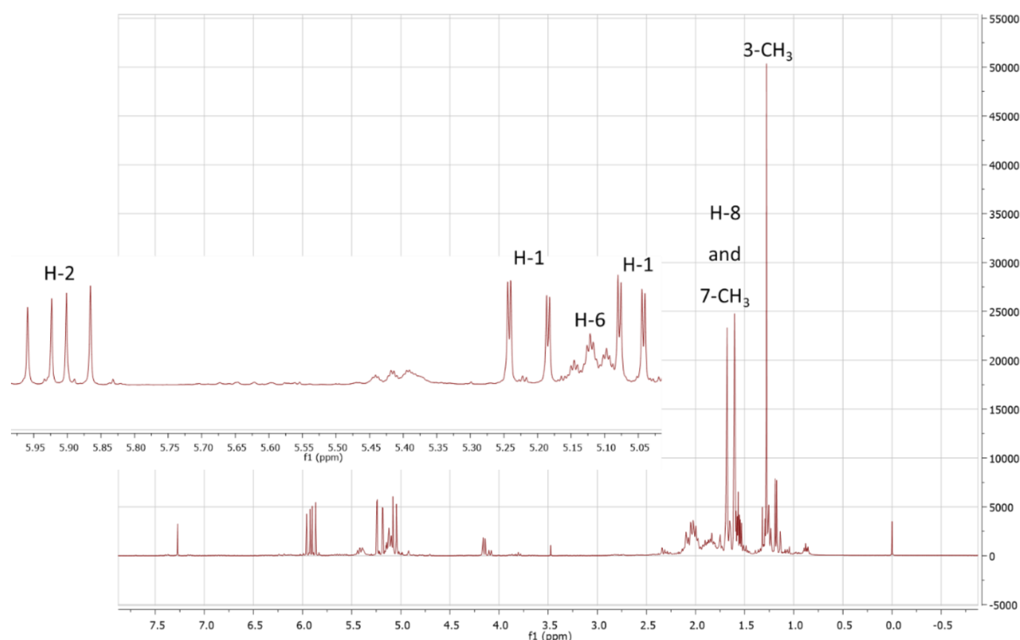

Figure 1. ^{1}H NMR Spectrum of *Solenostemma argel* Essential Oil.

Table 2. Monitoring Body Weight (g) of Rats during the Toxicity Study

	Control	EOSA		
		500 mg/kg	**1000 mg/kg**	**2000 mg/kg**
0 day	183 ± 1.224	177.6 ± 1.140	177.6 ± 0.547	178.4 ± 1.140
7th day	183.6 ± 4.393	185.6 ± 1.816	186.8 ± 1.303	186 ± 1.581
14th day	191.4 ± 2.880	186.8 ± 3.563	193.8 ± 3.563	192.8 ± 4.024

Table 3. Biochemical Parameters of Blood Serum during the Toxicity Study

	Control	EOSA		
		500 mg/kg	**1000 mg/kg**	**2000 mg/kg**
Urea (g/L)	0.446 ± 0.07	0.448 ± 0.06	0.484 ± 0.07	0.461 ± 0.07
Creatinine (mg/L)	11.306 ± 0.38	9.15 ± 1.28	9.390 ± 0.84	8.260 ± 1.71
ALP (U/L)	133.94 ± 6.92	94.358 ± 19.25	99.82 ± 27.14	101.44 ± 16.95
ALT (U/L)	51.78 ± 4.74[a]	68.694 ± 14.71	106.72 ± 45.98	128.38 ± 24.93[b]
AST (U/L)	62.16 ± 6.44[a]	84.4 ± 18.94	114.76 ± 24.09	146.34 ± 19.40[b]
Total protein (g/L)	70.18 ± 1.30	62.052 ± 6.16	56.242 ± 7.48	60.632 ± 8.76
Albumin (g/L)	31.54 ± 1.71	40.224 ± 4.28	33.244 ± 2.78	34.914 ± 3.16

Values are expressed as mean ± Standard deviation (n = 5). The numbers with the same letter show no significant difference (p>0.05).

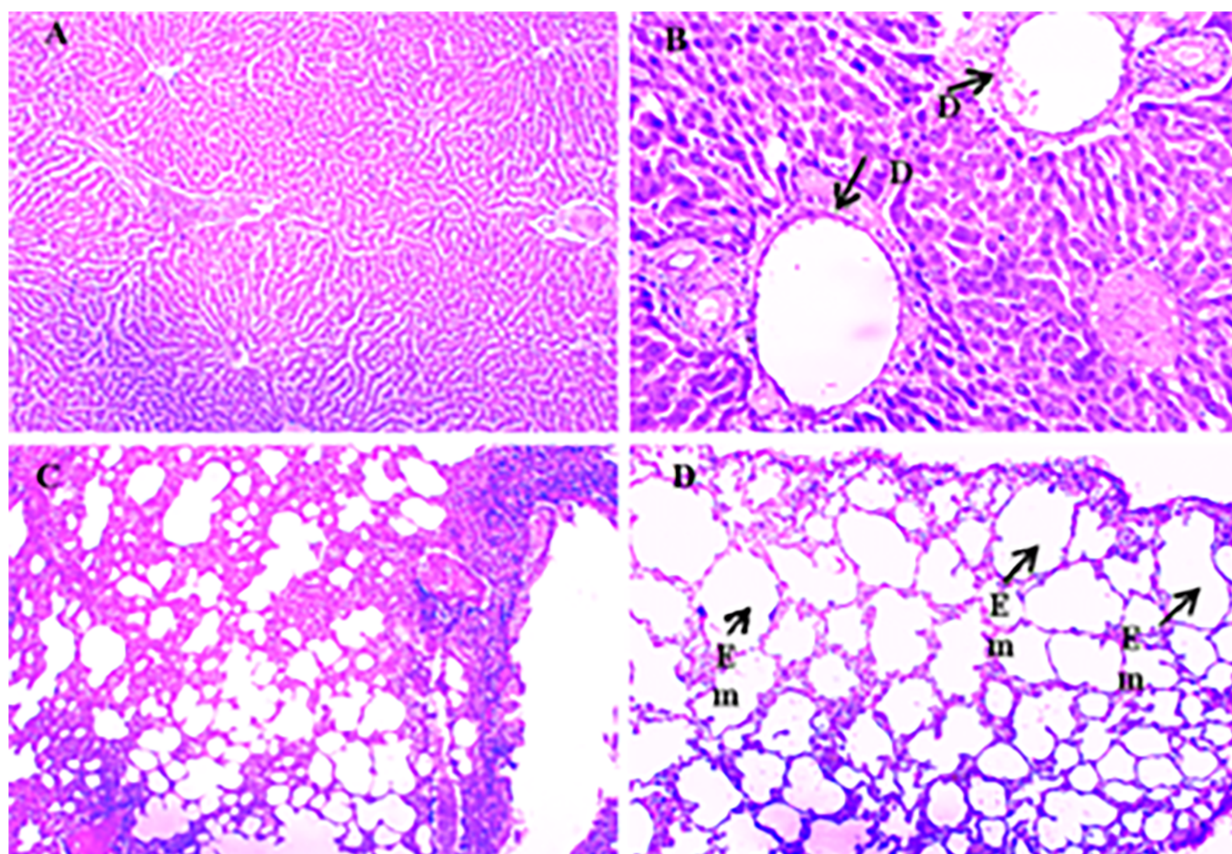

Figure 2. Histopathological Analysis of Liver and Lungs Treated with EOSA in the Acute Toxicity stained with Hematoxylin and Eosin Method (H&E). **(A)** Control: normal liver parenchyma x4; **(B)** EOSA 2000 mg/kg: focal venous stasis x10; **(C)** Control: normal lung parenchyma x10; **(D)** EOSA 1000 and 2000 mg/kg: peripheral emphysema x10. **D**: Dilatation, **Em**: Emphysema.

Furthermore, an anatomopathological examination of the lungs described discrete and slight pulmonary lesions in the form of oedematous alveolitis and peripheral emphysema observed at doses of 1000 and 2000 mg/kg (Figure 2). At the end, the anatomopathological examination of the kidneys, heart, and spleen unveiled the absence of toxic lesion for all the doses tested.

Analgesic Activity

Writhing Test in Mice

The effect of EOSA on peripheral pain is shown in Table 4. The oral administration of EOSA at doses of 250 and 400 mg/kg generated an important dose-dependent diminution in the number of writhing induced by intraperitoneal injection of acetic acid compared to the control group (p<0.0001), with an inhibition rate of cramps of 72.71 and 92.41%, respectively. In addition, the reference drug resulted in a significant analgesic effect similar to that of EOSA at a dose of 250 mg/kg.

Formalin Test

As illustrated in Figure 3, EOSA caused an important dose-dependent inhibition of both neurogenic and inflammatory phases of the formalin test at doses of 250 and 400 mg/kg compared to the control (p<0.0001). In the early phase, the percentage of inhibition recorded was 55.6 and 77.8% respectively, while for the last phase, the rate reached 61.1 and 88.9% respectively, considering the control group.

In this pain model, the recorded anti-nociceptive effects are significantly greater against the second phase. As expected, the standard drug morphine (10 mg/kg) produced a strong anti-nociceptive activity in both phases with an inhibition percentage of 100 and 88.9% in the early and last phases in that order. The protective property recorded in the

Table 4. Effect EOSA on Writhing Test in Mice

	Dose (mg/kg bw.)	Number of Writhes	%Inhibition
Control	(10 mg/kg b w.)	57.83 ± 4.79^{a}	-
Indomethacin	10	12.16 ± 1.83^{b}	78.93^{a}
EOSA	250	15.66 ± 2.33^{b}	72.71^{a}
EOSA	400	4.33 ± 5.16^{c}	92.41^{b}
		***	***

Values are expressed as mean ± Standard deviation (n = 6). The numbers with the same letter show no significant difference after analysis of one-way ANOVA followed by Tukey test. *** p<0.0001 compared to the control group.

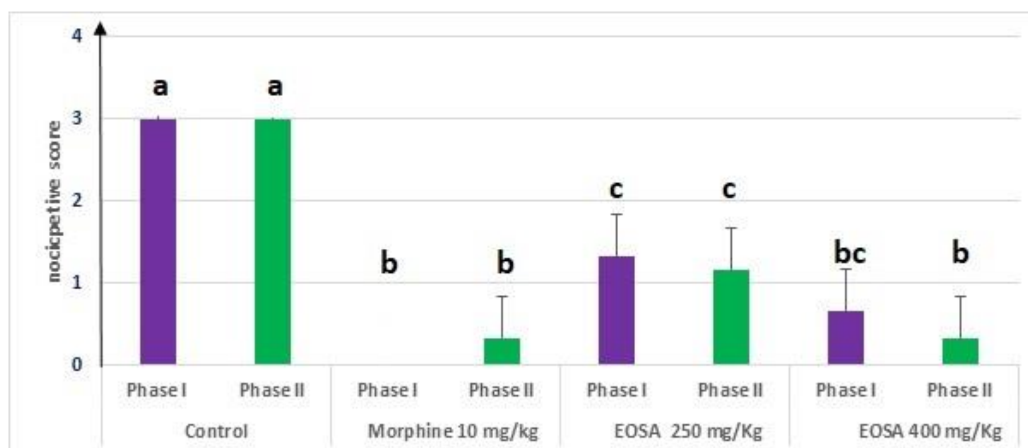

Figure 3. Effect of EOSA on Formalin Test in Rats. Nociceptive scores are expressed as mean ± Standard deviation (n = 6). The numbers with the same letter show no significant difference after analysis of one-way ANOVA followed by Tukey test.

Table 5. Effect of EOSA on Hot Plate Test in Mice

	Dose (mg/kg b w.)	Latency			
		$T_{30\ min}$	$T_{60\ min}$	$T_{90\ min}$	$T_{120\ min}$
Control	(10 mg/kg b w.)	5.38 ± 0.08^a	5.45 ± 0.05^a	5.34 ± 0.08^a	5.33 ± 0.08^a
Morphine	10	30.19 ± 0.14^b	30.90 ± 0.04^b	30.09 ± 0.04^b	30.05 ± 0.04^b
EOSA	250	12.44 ± 0.82^c	15.56 ± 0.26^c	9.34 ± 0.44^c	8.29 ± 0.48^c
EOSA	400	30.02 ± 1.24^b	30.38 ± 0.34^b	20.82 ± 1.42^d	17.84 ± 0.94^d

Values are expressed as mean ± SD (n = 6). The numbers with the same letter show no significant difference after analysis of one-way ANOVA followed by Tukey test. *** p<0.0001 compared to the control group.

last phase by EOSA at the dose of 400 mg/kg was similar to morphine (Figure 3).

Hot Plate Test

The result of the analgesic property of EOSA on central pain induced by the hot plate is shown in Table 5. It indicated that oral administration of the EOSA at doses of 250 and 400 mg/kg considerably attenuated the hot-plate thermal stimulation all along the time points studied (p<0.0001), considering the control group. The analgesic effect of EOSA at the dose of 400 mg/kg was comparable to the standard drug morphine (10 mg/kg) at measurement times of 30 and 60 min. In contrast, morphine substantially prolonged the hot-plate latency and produced a strong anti-nociceptive effect all through the time points studied compared to control and EOSA at doses of 250 and 400 mg/kg (p<0.0001).

EOSA prolonged the latency to nociceptive behaviour in the hot plate test, with a maximum inhibition percentage of 82.04% recorded at 60 min at a dose of 400 mg/kg comparable to that of morphine (82.33%) at the same time, suggesting that it has a very significant central analgesic effect.

Discussion

The GC-MS analysis of the essential oil of *S. argel* leaves allowed the identification of twenty components. Considering the general guidelines[14] indicating that a component is considered as major if it represents 20 to 70% of the components present in the mixture, linalool only can be considered as a major component representing more than 57% of the essential oil components of *S. argel*. Nevertheless, other components such as terpineol (12.954%) and trans-geraniol (12.658%) represent more than 10% of the mixture and can participate in the recorded biological effects. NMR analysis of the essential oil of *S. argel* has shown that the main compound is linalool. Generally, this major component determines the pharmaceutical activities of the essential oil.[15]

During the experimental period of acute toxicity of EOSA, no sign of morbidity or mortality was observed during the experiment indicating good apparent tolerance of the essential oil of *S. argel* by rats. Increased levels of AST and ALT recorded in rats treated at dose of 2000 mg/kg may be a result of liver toxicity. Effectively, increases in ALT and AST levels in serum are related to liver toxicity from hepatotoxic drugs.[16]

The evaluation of pathological damage in the organs, both macro and microscopically, is the basis of safety testing.[17] The results recorded after histological examination demonstrate the low oral toxicity of EOSA for the highest doses of 1000 and 2000 mg/kg.

The acetic acid writhing test is a sensitive test for both opioid and non-opioid analgesics, widely used for evaluating peripheral analgesics.[18] EOSA significantly inhibited the number of writhing responses in mice in all concentrations evaluated showing a significant analgesic effect. The pain induced by the injection of acetic acid generates the release of substance P, serotonin, histamine, bradykinin, and

prostaglandins (PGE2α, PGF2α) stimulating the peripheral nociceptive neurons and increasing the vascular permeability.[19,20] Therefore, EOSA probably acts by inhibition of one or more of these chemical mediators.

The formalin test is a pain model consisting of two different phases. The first phase (from 0 to 5 min after injection of formalin) results from direct stimulation of nociceptors. Substance P, glutamate, and bradykinin are described to participate in this phase, consisting of non-inflammatory pain. The second phase (from 15 to 30 min) is thought to be an inflammatory response with associated pain, a process in which several inflammatory mediators are involved, including histamine, serotonin, prostaglandins, and bradykinin.[21,22]

EOSA induced analgesic activity on both phases of the formalin test, indicating that both have a direct effect on the nociceptor and inflammatory pain inhibition. Data obtained in the formalin test agree with those from the hot plate and writhing tests. This effect of EOSA is probably due to the inhibition of the synthesis or action of kinin and prostaglandins or both.

The standard hot-plate test is based on the thermal stimulus-induced hyperalgesia specific for centrally mediated nociception.[21] The anti-nociceptive effect recorded confirms the activity on the central pain shown in the first phase of the formalin test and is probably mediated by inhibition of central pain receptors.

The anti-nociceptive effect of EOSA can be attributed to the action of its main compound, linalool, or to the synergistic action of other compounds. Several *in vivo* studies confirm the anti-nociceptive properties of linalool. Indeed, a previous study demonstrated the anti-nociceptive effect of linalool both on the inflammatory pain induced by acetic acid during the writhing test, and on neurogenic pain during the hotplate test.[23] They also demonstrated the anti-nociceptive effect of linalool on formalin-induced pain during formaldehyde testing phases.[24] The anti-nociceptive properties of linalool have been mainly attributed to a positive effect on the opioid, dopaminergic and muscarinic signal pathways as well as to the negative modulation of glutamatergic pathway.[25] In fact, the anti-nociceptive activity produced by linalool during the writhing test seems to depend both on cholinergic and opioidergic neuro-transmission, whereas, during the hot plate test, linalool appears to activate different mechanisms such as muscarinic transmission, via M2 receptors, opioidergic transmission and dopaminergic transmission, via D2 receptors as well as K + channels.[26]

Furthermore, terpineol present in EOSA may also participate in the anti-nociceptive effect recorded. Indeed, the previous studies reported the anti-nociceptive activity of terpineol on models of peripheral and central pain, suggesting anti-nociceptive effects associated with the release inhibition of substance P and other inflammatory molecules such as serotonin, histamine, bradykinin, and prostaglandins.[27,28] Terpineol can also inhibit the expression of pro-inflammatory cytokines and the activation of the NF-κB pathway.[29] In addition, geraniol, another component of EOSA, could contribute to the observed anti-nociceptive effect. Indeed, a previous study demonstrated the anti-nociceptive action of geraniol by two experimental models, the writhing test, and the formaldehyde test. Geraniol elicited a significant anti-nociceptive effect in the second phase of the formalin test and in the writhing test, suggesting that its anti-nociceptive property is mainly related to the inhibition of the inflammation and the peripheral pain by the release of pro-inflammatory mediators including bradykinin, prostaglandins, and serotonin.[30]

Conclusion

This study demonstrates for the first time the anti-nociceptive effects of *S. argel* essential oil on central and peripheral pain. Our data give scientific support to the use of *S. argel* in traditional medicine to cure pain. The safety and effectiveness of *S. argel* essential oil at the doses tested make it an interesting therapeutic agent against pain, alone or in combination with other analgesics. Our future study will be based on the possible mechanisms of action of this oil during the analgesic process.

Authors' Contributions

DKB was the main investigator participating at all parts of the work. BC participated to the writing and the reviewing of the manuscript. HA and SB participated to the chemical characterization of the EOSO. DCGAP supervised the chemical study. HB and KB were involved in the histopathologic studies. SZ performed the statistics. MHB was the supervisor of the work. All authors have read and approved the final manuscript.

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Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

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