—Regular Articles—

Antinociceptive, Anti-inflammatory and Antipyretic Effects of *Solanum nigrum* Chloroform Extract in Animal Models

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AIM: The present study was carried out to evaluate the antinociceptive, anti-inflammatory and antipyretic effects of chloroform extract of *Solanum nigrum* leaves using various animal models. METHODS: The extract was prepared by soaking (1 : 20; w/v) the air-dried powdered leaves (20 g) in chloroform for 72 hrs followed by evaporation (40°C) under reduced pressure to dryness (1.26 g) and then dissolved (1 : 50; w/v) in dimethylsulfoxide (DMSO). The supernatant, considered as the stock solution with dose of 200 mg/kg, was diluted using DMSO to 20 and 100 mg/kg, and all doses were administered (*s.c.*; 10 ml/kg) in mice/rats 30 min prior to tests. RESULTS: The extract exhibited significant ($p<0.05$) antinociceptive activity when assessed using the abdominal constriction, hot plate and formalin tests. The extract also produced significant ($p<0.05$) anti-inflammatory and antipyretic activities when assessed using the carrageenan-induced paw edema and brewer's yeast-induced pyrexia tests. Overall, the activities occurred in a dose-independent manner. CONCLUSION: The present study demonstrated that the lipid-soluble extract of *S. nigrum* leaves possessed antinociceptive, anti-inflammatory and anti-pyretic properties and confirmed the traditional claims.

Key words——*Solanum nigrum*; chloroform extract; antinociceptive; anti-inflammatory; antipyretic

INTRODUCTION

Solanum nigrum L., which belongs to the Solanaceae family, has been used traditionally to treat various ailments such as pain, inflammation and fever.[1,2] Locally known as '*Pokok Ranti*', the plant has been reported to possess analgesic, antiperiodic, antiphlogistic, diuretic, purgative and sedative effects. The leaves, stem and roots are used as a poultice or wash to treat cancerous sores, boiled, leucoderma and wounds while extracts of the plant are claimed to possess anti-inflammatory, antispasmodic and vasodilator effects.[1] There is also report on the used of the plant in the manufacture of locally analgesic ointments and the fruits, in particular, have been used as an analgesic for toothaches. The bruised fresh leaves of *S. nigrum* have been used externally to ease pain, abate inflammation, and reduce fever.[1] The pain relieving effect is believed to be attributed to its greater narcotic properties. The Arabs, for example, used the leaves to treat burns and ulcers. The juice of *S. nigrum* leaves has also been used in the treatment of earache, gout and ringworm, and when mixed with vinegar, is said to be good as a mouthwash and gargle.[2] Although there is a lot of disagreement over whether the leaves or fruits of *S. nigrum* are poisonous or not, it is believed that the toxic effects vary considerably according to the part or cultivar of the plant being used or grown. For example, the unripe fruits of *S. nigrum* contain the highest concentration of toxin, particularly solanine.[1]

Scientifically, most pharmacological studies reported on *S. nigrum* focused on the antitumour activity[3—13] with several reports on the antiulcerogenic and ulcer healing,[14] hepatoprotective,[15,16] cytoprotective,[17] antioxidative,[18,19] antimicrobial,[20] molluscicidal, larvicidal and cercaricidal[21—23] and centrally-mediated depressant[24] activities. Several bioactive compounds have been isolated and identified from the whole plant of *S. nigrum*. Ikeda et al.[25] have reported on the present of two new steroidal oligoglycosides, namely nigrumnins I and II while Hu et al.[26] have earlier reported on the identification of three known steroidal glycosides, namely β-2-solamargine, solamargine and degalactotigonin. Hu et al.[26] also reported on the antineoplastic activity of

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the identified compounds. As mentioned earlier, all of the studies related to the antitumour activity of *S. nigrum* are attributed to the identified 150-kDa glycoprotein, which contain high contents of glycine and proline.

Based on the literature findings, no scientific reports on the antinociceptive, anti-inflammatory and antipyretic effects of any parts of *S. nigrum* have been documented. By focusing on the leaves of *S. nigrum*, at the moment, the aim of the present study was to investigate on the potential antinociceptive, anti-inflammatory and antipyretic effects of its aqueous extract using various animal models.

MATERIALS AND METHODS

Plant Material The leaves of *S. nigrum* were bought from the local market in Shah Alam, Selangor, Malaysia in July-August, 2005 and a voucher specimen (SK 1125/06) was deposited at the Herbarium of the Laboratory of Natural Products, Institute of Bioscience, UPM, Serdang, Selangor, Malaysia.

Preparation of *Solanum nigrum* Chloroform Extract (CESN) The CESN was prepared by soaking the air-dried powdered leaves of *S. nigrum* (20 mg) in chloroform in the ratio of 1 : 20 (w/v) for 72 hrs. The supernatant was collected and filtered using Whatman No. 1 filter paper while the remaining plant residue was discarded. The filtered supernatant obtained was evaporated to dryness and the weight of the crude dried chloroform extract obtained was measured (1.26 g). The dried extract was diluted in dimethyl sulfoxide (DMSO) (1 : 50; w/v) and considered as the stock solution with dose of 200 mg/kg. The stock solution was diluted with DMSO to the doses of 20 and 100 mg/kg for antinociceptive, anti-inflammatory and antipyretic studies.

Preparation of Drugs The dosages of acetylsalicylic acid (ASA; Bayer, Singapore) and morphine sulphate (Sigma, U.S.A.), used as reference drugs, were chosen based on previous reports.[27,28]

Experimental Animals Male Balb/C mice (20—30 g) and Sprague-Dawley rats (200—300 g) were obtained from the Institute of Medical Research, Kuala Lumpur, Malaysia. The animals were transported to the Animal Unit of Faculty of Veterinary, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia and acclimatized for 1 week before used. During the acclimatization period, the animals were supplied with a standard commercial diet and water *ad libitum* and kept in rooms maintained at 27±2°C, 70—80% humidity and 12 h light/darkness cycle. The experimental procedures were carried out in accordance with the Animal Ethics Committee rules and regulations followed by the University and the ethical guidelines for investigations of experimental pain in conscious animals.[29]

All mice were equally divided into 12 groups of 7 mice each ($n=7$) and received (*sc*) dH_2O, DMSO, ASA (100 mg/kg) or CESN (20, 100 and 200 mg/kg) 30 min prior to subjection to the abdominal constriction or hot plate tests, respectively. On the other hand, all rats were equally divided into 19 groups of 5 rats each ($n=5$). The first seven groups were used in the formalin test and received (*sc*) dH_2O, DMSO, 100 mg/kg ASA, 5 mg/kg morphine or CESN (20, 100 and 200 mg/kg), respectively 30 min prior to subjection to the said test. The second and third six groups were used in the anti-inflammatory and antipyretic studies, and received (*sc*) dH_2O, DMSO, 100 mg/kg ASA or CESN (20, 100 and 200 mg/kg), respectively 30 min prior to subjection to the said tests. All of the test solutions were administered in the volume of 10 ml/kg body weight.

Antinociceptive Assay

Abdominal Constriction Test The abdominal constriction test[30] was used as described by Zakaria et al.[27] to assess the chemical-induced peripheral antinociceptive activity of CESN.

Hot Plate Test The 50°C hot-plate test[31] with slight modification as described by Zakaria et al.[32] was used to assess the thermal-induced central antinociceptive activity of CESN. The latency to a discomfort reaction (licking of the paws or jumping) was recorded at 0.5, 1, 2, 3, 4 and 5 hrs following the *sc* administration of the respective dH_2O, ASA or CESN.

Formalin Test The formalin test[33] with slight modifications was used to assess the antinociceptive effect of CESN at different phases of nociception. Pain was induced by administration of 50 μl of 5% formalin in the subplantar region of the left hind paw. Rats were injected (*sc*) with the respective dH_2O, DMSO, ASA or CESN 30 min prior to the formalin injection. The rats were individually placed in transparent Plexiglass cage observation chamber and the amount of time spent licking the injected paw, considered as an indicator of pain, was recorded for duration of 30 min following the formalin injection.

The early phase of nociception was measured between 0—5 minutes while the late phase of nociception was measured 15—30 minutes after formalin administration.

Anti-inflammatory Assay The carrageenan-induced paw edema test[34] with slight modification as described in Zakaria et al.[35] was used to assess the anti-inflammatory effect of CESN. The thickness of paw was measured using the caliper before (BF) and after carrageenan (*ip*) treatment at 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hrs. The results obtained for the ASA- or CESN-treated groups were compared with the dH_2O-treated group and the difference between the readings was taken as the indicator of edema.

Antipyretic Assay The brewer's yeast (BY)-induced pyrexia test[36] but with slight modifications was used as described by Zakaria et al.[35] to assess the antipyretic activity of CESN. The rectal temperature of each rat was measured before (BF) and after BY treatment at 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 hrs using a digital thermometer (SK-1250MC, Sato Keiryoki Mfg. Co., Ltd., Japan).

Statistical Analysis The results are presented as Mean ±Standard Error of Mean (S.E.M.). The one-way ANOVA test with Dunnett *post-hoc* test was used to analyze and compare the data, with $p<0.05$ as the limit of significance.

RESULTS

Figure 1 shows the antinociceptive effect of CESN assessed using the abdominal constriction test in mice. Unexpectedly, the extract exhibited significant ($p<0.05$) antinociceptive activity in a dose-independent manner. The 20 and 200 mg/kg CESN produced antinociceptive levels of more than 80% while the 100 mg/kg CESN exhibited total lost of antinociceptive activity. Interestingly, the first two doses that showed an antinociceptive activity were found to produce antinociceptive levels that are higher than that of 100 mg/kg ASA. In addition, DMSO caused significant ($p<0.05$) reduction in the number of abdominal constrictions.

Figure 2 shows the antinociceptive effect of CESN assessed using the hot plate test in mice. The extract exhibited a significant ($p<0.05$) antinociceptive activity until the end of the experiment, which is, however, less effective when compared to the 5 mg/kg morphine. The activity was also observed in a dose-independent manner with equieffective antinociceptive activity seen in all doses of CESN used between the interval times of 0.5 to 2 hrs. At the interval time of 3 hrs, the 100 and 200 mg/kg CESN activity were found to decrease remarkably compared to the 20 mg/kg CESN. However, their activities became equieffective again for the last 2 hrs of the experiment times. Except at the 0.5 hr time interval where it produced no significant activity, 5 mg/kg morphine was found to produce remarkable activity when compared to the extract for the next 5 hrs. DMSO did not cause any significant change in the latency to discom-

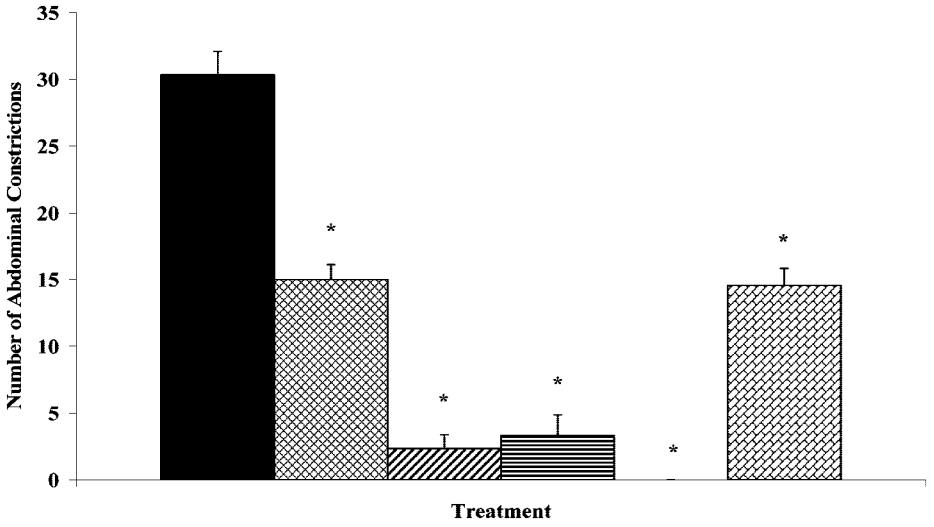

Fig. 1. The Antinociceptive Effect of CESN Assessed by the Abdominal Constriction Test in Mice

*Data differ significantly ($p<0.05$) when compared against the dH_2O-treated group. ■ dH_2O, ▩ 100 mg/kg ASA, ▨ 20 mg/kg CESN, ▤ 100 mg/kg CESN, □ 200 mg/kg CESN, ▨ DMSO.

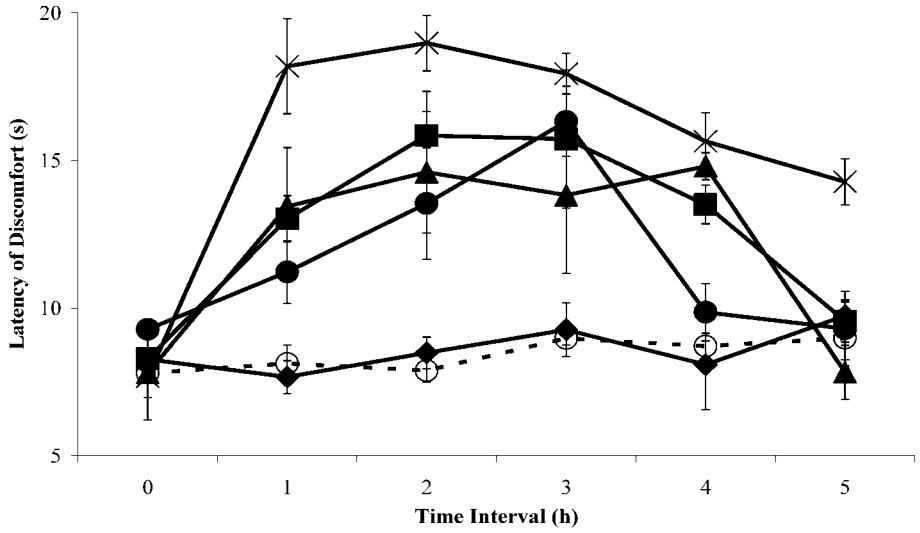

Fig. 2. The Antinociceptive Effect of CESN Assessed by the Hot Plate Test in Mice
dH_2O, 5 mg/kg Morphine, 20 mg/kg CESN, 100 mg/kg CESN, 200 mg/kg CESN, DMSO.

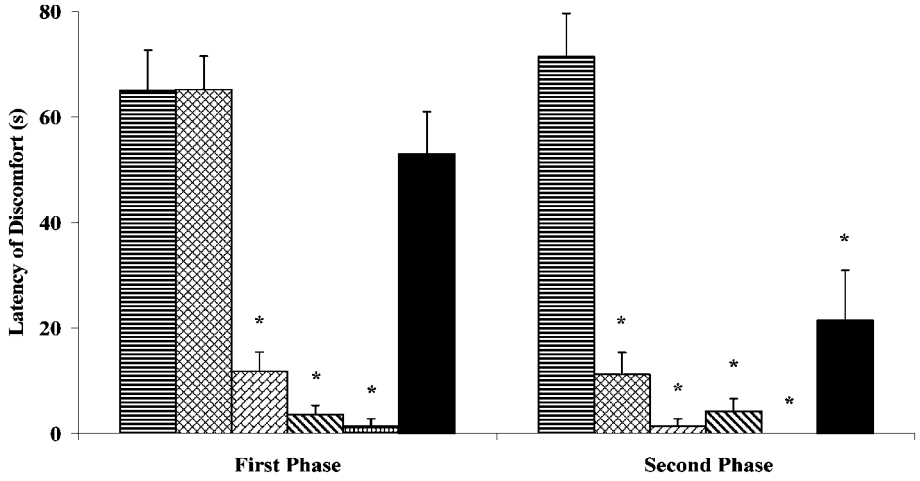

Fig. 3. The Antinociceptive Effect of CESN Assessed by the Formalin Test in Rats
*Data differ significantly ($p<0.05$) when compared against the dH_2O-treated group. dH_2O, 100 mg/kg ASA, 20 mg/kg CESN, 100 mg/kg CESN, 200 mg/kg CESN, DMSO.

fort until the end of the experiment.

The antinociceptive effect of CESN assessed using the formalin test in rats was shown in Fig 3. The extract was found to produce significant ($p<0.05$) antinociceptive in the early and late phases of the formalin-induced nociception. Although the activity also occurred in a dose-independent manner, unlike the effect seen in the abdominal constriction test, the CESN-induced antinociceptive effect can be seen at all of the doses used. DMSO was found to produce significant ($p<0.05$) effect only in the late phase of the formalin test.

Figure 4 shows the anti-inflammatory effect of CESN assessed using the carrageenan-induced paw edema test in rats. Unexpectedly, the CESN produced significant ($p<0.05$) anti-inflammatory activity, in what appeared to be as a two phases anti-inflammatory activity. The short first phase of anti-inflammatory activity occurred 1 to 2 hrs after the carrageenan administration before it diminished at the third hours of the time interval. This activity was found to appear again, in what could be said as second phase of anti-inflammatory activity, after 4 hrs, became significant ($p<0.05$) between 5 to 6 hrs and diminished after 7 hrs of the carrageenan administration. DMSO caused significant ($p<0.05$) change in the thickness of the

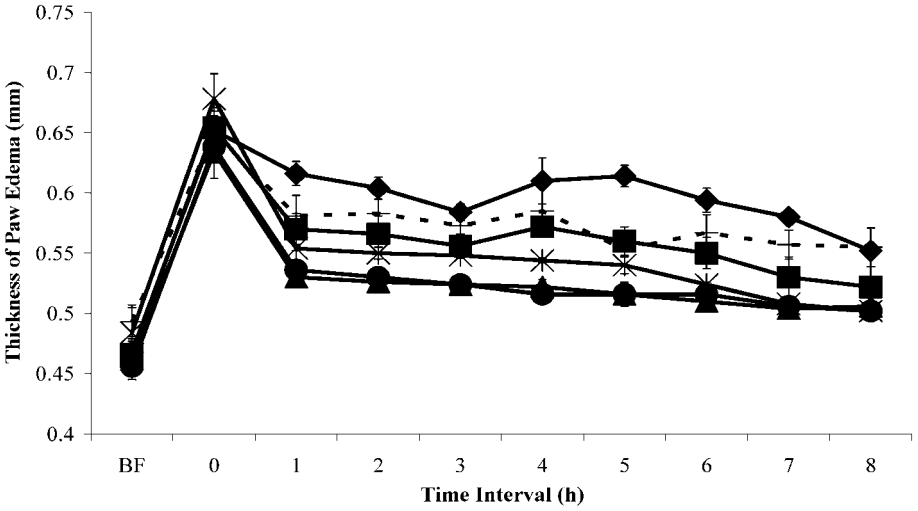

Fig. 4. The Anti-inflammatory Effect of CESN Assessed by the Carrageenan-induced Paw Edema Test in Rats
dH_2O, 100 mg/kg ASA, 10% CESN, 100 mg/kg CESN, 200 mg/kg CESN, DMSO.

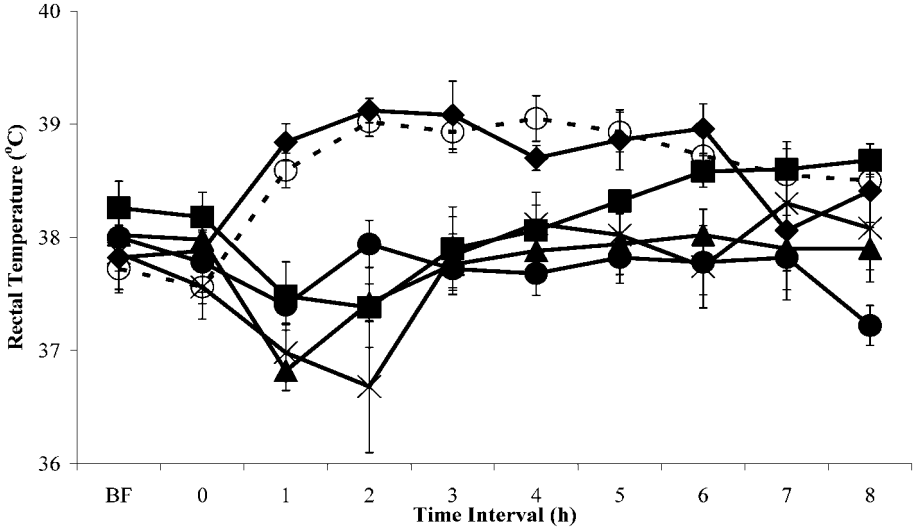

Fig. 5. The Antipyretic Effect of CESN Assessed by the BY-induced Pyrexia Test in Rats
dH_2O, 100 mg/kg ASA, 20% CESN, 100 mg/kg CESN, 200 mg/kg CESN, DMSO.

edematous paw only at the interval time of 1 and 5 hrs indicating slight anti-inflammatory activity.

Figure 5 shows the antipyretic effect of CESN assessed using the BY-induced pyrexia test in rats. The extract, at all doses, exhibited significant ($p<0.05$) antipyretic activity for the first 3 hrs after the BY administration. Except for the 200 mg/kg CESN activity, which can be observed until the 5 hrs time interval, the activity of 20 and 100 mg/kg CESN was found to diminish after 4 hrs of the BY administration until the end of the experiment. On the other hand, 100 mg/kg ASA exhibited significant ($p<0.05$) antipyretic activity only for the first 6 hrs after the BY administration that is greater than the CESN. DMSO did not influence the rectal temperature of rats indicating lacks of antipyretic activity.

DISCUSSION

In the present study, the chloroform extract of *S. nigrum* was found to exhibit remarkable antinociceptive, anti-inflammatory and antipyretic activities in a dose-independent manner. The observed antinociceptive activity could probably be due to its claimed narcotic properties.

It is believed that the antinociceptive activity of CESN is mediated at the peripheral and central level

since the extract was found to produce an activity in all antinociceptive assays used in a way shown by morphine, but not ASA. Morphine, a centrally acting drug, has been shown to show antinociceptive activity in the abdominal constriction,[37] hot plate[28] and formalin tests.[38] Since the abdominal constriction test was not specific as it did not indicate whether the activity was due to peripheral and/or central activity[39] additional experiments, for examples hot plate and formalin tests, are required before the actual mechanism of analgesic compounds could be draw. According to Pini et al.[40] the hot plate test, which used thermal stimulus to produce an acute, non-inflammatory, nociception, is a good model for studying central antinociceptive activity. On the other hand, the formalin test, which exhibited biphasic nociceptive effect, is used to study the antinociceptive, non-anti-inflammatory effects of drugs.[41] The biphasic effect, labeled as early and late phases, is due to direct neurogenic stimulation and tissue-mediated response, respectively.[42]

Furthermore, it is also plausible to suggest that part of the mechanism of antinociceptive activity observed in CESN involved blocking of the peripheral cyclooxygenase (COX) enzyme[43] activity. This suggestion is based on report made by Berkenkopf and Weichman[44] that the acetic acid induce abdominal constriction by causing the release of prostacyclin, which in turn is synthesized by the COX, within the abdominal cavity of the animal. The blocking of central COX activity[43] by CESN is also believed to take place based on Pini et al.[40] report that paracetamol, often classified as an NSAID, exerted its central antinociceptive effect as assessed using the hot plate test *via* inhibition of the central COX.[45] The CESN showed a characteristic of strong analgesics like opioid agonists since it blocked both the chemically- and thermally-induced nociceptive action, as well as the early and late phases of the formalin test.[33,46] Although not yet proven, it is plausible to associate the centrally-mediated depressant activity demonstrated by Perez et al.[24] with the antinociceptive activity of *S. nigrum*.

The anti-inflammatory activity of CESN is suggested to involve inhibition of COX activity based on claims that the carrageenan-induced inflammatory edema model is a cyclooxygenase (COX)-dependent reaction[47] and is more effectively controlled with arachidonate cyclooxygenase, but not arachidonate lipooxygenase, inhibitors.[48] Furthermore, the anti-inflammatory activity of *S. nigrum* could also be associated with the plant antioxidant activity[18,19] since inflammatory activity has been demonstrated to be accompanied by elevation in the free radicals level.[49] On the other hand, the antipyretic activity of CESN is believed to be due to the ability of the lipid-soluble compounds to cross the blood-brain barrier[50,51] and inhibit the central COX activity.[43]

The present study has demonstrated that the 100% DMSO, used to dissolve the CESN, caused significant changes only in the abdominal constriction, late phase of the formalin and paw edema tests when given alone in all assays used, which is against the report made by Fadeyi et al.[52] DMSO have also been reported to produce insignificant analgesic or anti-inflammatory activity when given *via* the systemic route.[53] However, since the number of constrictions, latency of pain and diameter of edematous paw reduced were significantly higher than that of the extracts, it is plausible to suggest that the antinociceptive and anti-inflammatory activities observed in those tests were due to the CEMM antinociceptive and anti-inflammatory properties. This suggestion seems to be supported by the fact that the extract also produced antinociceptive and antipyretic activities when assessed against the hot plate and BY-induced pyrexia tests. This finding also suggested that DMSO does not contribute to the CEMM ability to exhibit those activities. The conflicting reports cited above for the anti-inflammatory and analgesic properties of DMSO are believed to be partially dependent upon the types of animals, experimental models and methods used to measure those parameters.

Several types of bioactive compounds, isolated from *S. nigrum*, could be linked to the CESN observed activities. For example, the 150-kDa glycoprotein has been shown to stimulate apoptosis partly *via* reduction of nitric oxide (NO) production in HCT-116 cells[13] and NO have been associated with antinociceptive[54,55] and anti-inflammatory[56,57] activities. The present of steroidal glycosides[26] and steroidal oligoglycosides[25] could also contribute to the activities seen in CESN. Based on the data obtained, our findings have confirmed folklore uses of the plant as antinociceptive, anti-inflammatory and antipyretic agents.

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