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Assessment of analgesic and anti-inflammatory properties of crude extracts of ray fish, *Narcine brunnea*

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ABSTRACT

Objective: To assess the analgesic and anti-inflammatory properties of the crude petroleum ether and ether extracts of *Narcine brunnea*. **Methods:** The homogenized flesh was extracted exhaustively in a soxhlet apparatus separately with petroleum ether and ether. The Chemical analysis of petroleum ether and ether extracts was carried out by UV-VIS spectrophotometer, FT-IR and GC-MS. The analgesic and anti-inflammatory properties of the crude extract was assessed by hot plate, Haffner's tail clip and carrageenan induced rat paw oedema methods in animal models. **Results:** The GC-MS and EIMS revealed five compounds *viz.* 3, 5- dihydroxy phenyl acetic acid, phtha1ic acid, N-methyl 2, 3-dihydro 3-but-2-enyl indole 5-sulphonic acid, 2-methoxy serotonin sulphate and 3-but-2 enyl-indole-5- sulphonic acid. The results (mean± SE) of hot plate showed that the crude petroleum ether and ether extracts exhibited increase in basal reaction time from 2.150±0.043 and 2.300±0.058 at 0 min to 6.102±0.037 and 8.783±0.070 at 120 min respectively. The tail clip method revealed a well marked increase in basal reaction time of 6.817±0.031 in petroleum ether and 8.852±0.043 in ether extract at 120 min. The crude petroleum ether inhibited the oedema volume of 51% with a mean oedema volume of 3.465±0.022 at 4h, where as the crude ether extract produced to the extent of 56% inhibition of oedema volume with a mean 3.363±0.023 at 4 h. **Conclusions:** This study confirmed the analgesic and anti-inflammatory properties of *Narcine brunnea* observed during the ethno-pharmacological survey. In order to go towards a valuation of this traditional knowledge, further studies like purification, isolation and NMR must be carried out to determine which of these compounds are actually responsible for such properties.

1. Introduction

According to the World Health Organization, an estimate over 80% of the population of developing countries uses traditional medicines[1]. Traditional healing practices are as old as the advent of man; and are highly varied because it is ethnic, community specific and ecosystem specific. The traditional knowledge regarding the medicinal properties of fishes is prevalent among the fisherman community. *Narcine brunnea* commonly known as brown electric ray belongs to Elasmobranchii are traditionally used to treat the inflammatory disease and arthritis. A pudding of fish flesh is given to person suffering from arthritis. Our ethno-pharmacological survey based on Foundation for Revitalization of Local Health Traditions (FRLHT), Banglore, India among the tradipractitioners of fisherman community confirms these uses. However, there are as yet no published reports concerning the ethno-pharmacological use. The present study was aimed to assess the analgesic and anti-inflammatory properties of the crude petroleum ether and ether extracts of *N. brunnea* by using various experimental models in rodents and characterization of bioactive compounds.

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2. Material and methods

2.1. Fish collection

Fresh fishes were collected directly from fishing vessels of Puducherry coastal waters (11^0 46′ and 12^0 03′ N; 79^0 36 and 79^0 53′ E). Fish was identified as *N. brunnea* using the keys given by Ramaiyan and Sivakumar[2].

2.2. Preparation of extract

About 500 g of fresh fish was collected and homogenized at room temperature. The homogenized flesh was extracted exhaustively in a soxhlet apparatus separately with petroleum ether and diethyl ether for 4–6 h and left to cool over night[3]. Then the extracts were concentrated in vacuum to yield a thick, viscous, dark reddish brown mass (16g). The crude extract obtained in each case was solublished in DMSO before being fed to the mice and rats.

2.3. Experimental animal setup

Male albino rats (150–175 g) and male albino Swiss mice (25–30 g) were procured from College of Pharmacy, Mother Theresa Institute of Health Sciences, Puducherry used in the experiment. The animals were kept at room temperature and maintained in a 12 h light/ dark cycle and fed *ad libitum* with standard food and water. They were fasted for 24h before the experiment. All the test doses were administered intra peritoneal (*i.p*) which are 10 times lower than LD_{50} dose. All experimental procedures followed the guidelines on ethical standards for investigations and were carried out according to a protocol approved by the local Animal Ethics Committee in compliance with National and International rules on care and use of laboratory animals[4].

2.4. Chemical and spectral analysis

The Chemical analysis of petroleum ether and diethyl ether extracts was carried out to ascertain the aliphatic or aromatic, saturated or unsaturated nature of the constituents. UV spectrum was recorded in UV–VIS spectrophotometer (SHIMADZU–160, Japan). The elucidation and molecular mass of the compounds from crude extracts were performed on a GC–MS (SHIMADZU –QP 5000, Japan). About 2 mL of methanol dissolved sample was injected into a CBP–1 packed column (25mm x 0.25mm dia), the temperature increased linearly from 50 °C to 320 °C and the carrier gas pressure fixed at 79.80 Kpa for all the samples. Electron impact mass spectra were recorded for each compound separated in succession by GC, the relative intensities corresponding to their Rt of the molecular ion peak and the fragmented ion peaks were normalized with respect to the base peak. FT–IR spectrum was recorded between 4 000 cm^{-1} and 600 cm^{-1} for all the extracts using FT–IR (BRUKER IFS 85, Germany).

2.5. Analgesic effect– Hot plate method

The analgesic activity was assessed by hot plate (thermal) method as described by Woolfe and Macdonald[5]. The mice were divided into four experimental groups of six animals each. Group 1 served as control received normal saline (2 mL/kg). Group 2 was treated with standard drug (reference) buprenorphine (5 mg/kg) and group 3 and 4 were administered crude petroleum ether (65 mg/kg) and ether extracts (76 mg/kg) respectively. The animals were placed on a hot plate (Analgesiometer, Techno, India) maintained at a temperature of (55±0.5) °C. The basal reaction time, when the animals licked their paw or jumping occurred was recorded by a stop watch before 0, 15, 30, 60, 90, and 120 min after administration of crude extracts. A cut off time of 15sec was used. The increase in reaction time against control was calculated.

2.6. Analgesic effect– Haffner's tail clip method

Haffner's tail clip method was performed as described by Bartoszyk and Wild[6]. Group 1 to 4 received the control, standard drug (reference) and test extracts as in hot plate method. Artery clip with thin rubber sleeves was applied at the base of the animal tail. The time taken by the animal to make an effort to dislodge the clip was recorded before 0, 15, 30, 60, 90 and 120 min after administration of crude extracts. The increase in reaction time against control was recorded.

2.7. Anti–inflammatory activity – Carrageenan induced rat paw oedema method

The carrageenan– induced paw oedema assay was carried out in male albino rat (150–170 g) described by Winter *et al*[7]. Oedema was induced by sub–planter injection of 0.1 mL of freshly prepared 1% carrageenan (w/v) into the right hind paw of the rats of four groups of six animals each. Group 1 served as carrageenan control (0.1ml/kg), Group 2 was treated with standard drug (reference) diclofenac sodium (10 mg/kg). Group 3 and 4 were given crude petroleum ether (65 mg/kg) and ether extract (76 mg/kg) of *N. brunnea* respectively. The volume of pedal oedema was measured at 0, 1/2, 1, 2, 3 and 4 h after injection of carrageenan using a plethysmometer (Ugo Basile). All the treatment was given 30min prior to the injection of carrageenan. The percentage of oedema inhibition was calculated for each animal group.

2.8. Statistical analysis

The analyses of variance, ANOVA followed by Fisher test were used for statistical analysis. Data were expressed as mean±SE. A probability value ($P<0.05$) was considered significant.

3. Results

The results of the elemental analysis indicated the presence of carbon, hydrogen, oxygen and the absence of nitrogen and sulphur. The functional group analysis gave positive tests for the presence of aromatic, saturated acid and ester groups. The aromatic nature of the compounds was derived from the sooty flame produced by the concentrate of the extract. The absorption maxima at 277.5 nm appearing in the UV spectrum substantiated the aromatic nature of the compounds.

The crude petroleum ether extract of *N. brunnea* (CPEENB) exhibited strong IR signals appearing at 3 354 cm^{-1} due to O–H stretching, C=O and C=C stretching at 1 634 cm^{-1}, C–H asymmetric deformation at 1 489cm^{-1}, O–H bending at 1 195cm^{-1} and C–O stretching at 1045 cm^{-1} indicates the presence of OH, COOH and C=C groups. Two compounds could be resolved in GC–MS corresponding to the two peaks with Rt 15.011–16.395 and 23.375–25.511 min). The positive EIMS and their fragmentation pattern were recorded for 2 compounds separated by GC. The compound 1 corresponding the first peak gave the molecular ion peak at m/z 169 agreed with the molecular formula $C_8H_8O_4$. The characteristic fragmentation pattern suggested the compound as 3, 5– dihydroxy phenyl acetic acid (Figure 1). Compound 2 corresponding to the second peak of GC produced the molecular ion peak at m/z 167 inferred from the EIMS was in accordance with $C_8H_6O_4$ as phthalic acid (Figure 2).

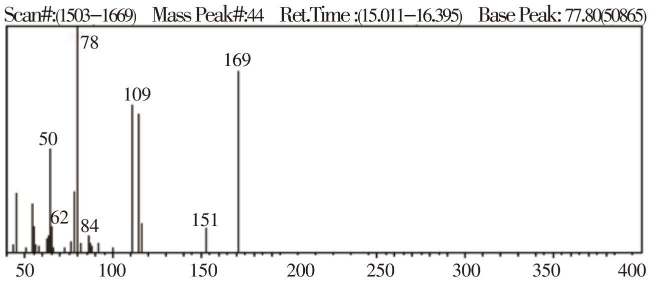

Figure 1. EIMS of compound 1 of crude petroleum ether extract of *N. brunnea.*

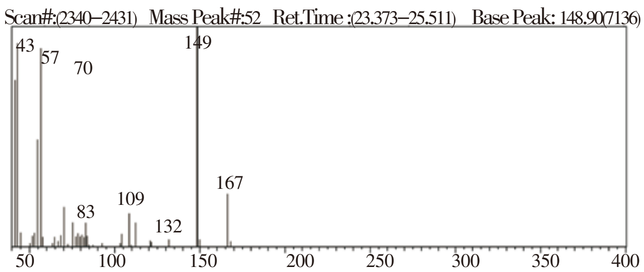

Figure 2. EIMS of compound 2 of crude petroleum ether extract of *N. brunnea.*

The elemental analysis of crude ether extract of *N. brunnea* (CEENB) discloses the presence of carbon, hydrogen, oxygen, nitrogen and sulphur. The functional group analysis revealed the presence of sulphonic acid and amine groups in an aromatic system, further evidenced by UV spectrum showed an absorption maxima at 259 nm indicating the aromatic nature of the compound.

The broad band at 3 347 cm^{-1} of FT-1R spectrum could be assigned to N-H and O-H stretching while 1 619, 1 398, 933 and 897 cm^{-1} respectively corresponded to N-H bending. C = O and C-N stretching appeared at 1 452 and 1 336 cm^{-1}. All these characteristics of the FT-IR spectrum of the extract together with the broad band at 1 191cm^{-1} were in favor of the presence of sulphonic acid and amine groups .The GC-MS exhibited 3 peaks with the Rt from 22.350 to 24.550. The fragmentation pattern that resulted from the EIMS of compound 1 contained the molecular ion peak at m/z 265 corresponding to the molecular formula $C_{13}H_{15}$ NO_3S and the fragment ion peaks at m/z 248, 164 and 105 suggested the compound to be N-methyl 2, 3-dihydro 3-but-2-enyl indole 5-sulphonic acid (Figure 3). EIMS of compound 2 exhibited a molecular ion peak at m/z 286 with the molecular formula $C_{11}H_{14}N_2O_5S$. The fragmentation pattern revealed the compound might be 2-methoxy serotonin sulphate (Figure 4). The molecular ion peak at m/z 251 observed in the positive EIMS of compound 3 was compatible with the molecular formula $C_{12}H_{13}NO_3S$. Based on the fragmentation pattern the compound was characterized as 3-but-2 enyl-indole-5- sulphonic acid (Figure 5). All the compounds have been systematically characterized and meticulously compared with those compounds already reported in herring, mackerel, cod liver oil and shark liver oil[8].

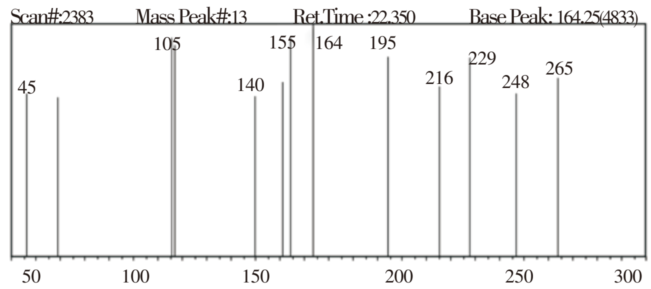

Figure 3. EIMS of compound 1 of crude ether extract of *N. brunnea.*

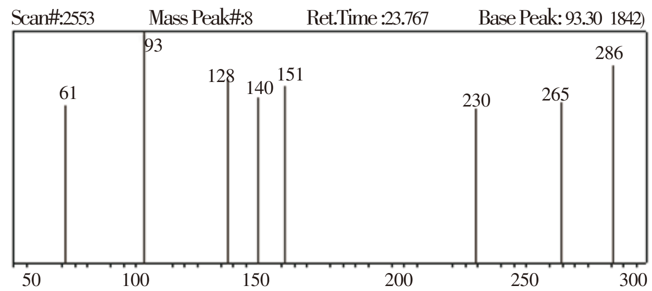

Figure 4. EIMS of compound 2 of crude ether extract of *N. brunnea.*

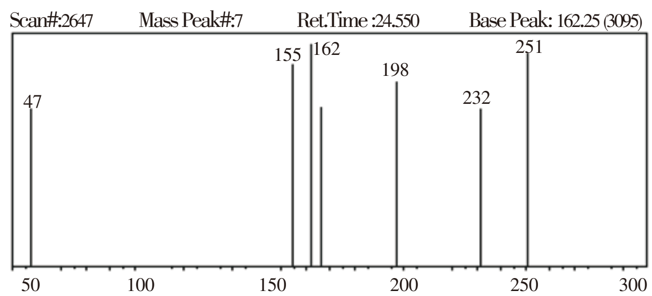

Figure 5. EIMS of compound 3 of crude ether extract of *N. brunnea.*

The results (mean±SE) of hot plate showed that the crude petroleum ether and ether extract exhibited increase in basal reaction time from 2.150±0.043 and 2.300±0.058 at 0 min to 6.102±0.037 and 8.783±0.070 at 120 min respectively (Figure 6). The tail clip method revealed a well marked increase in basal reaction time of 6.817±0.031 in petroleum ether and 8.852±0.043 in ether extract at 120 min (Figure 6), The basal reaction time of control groups were 2.233±0.061 and 2.667±0.033 in hot plate and tail clip methods respectively. Where as the standard drug (reference), buprenorphine showed maximum reaction time of 15.000±0.000 in both the models at 120 min.

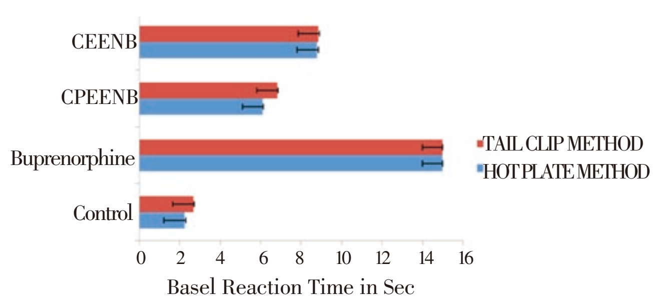

Figure 6. Analgesic activity of the crude petroleum ether and ether extract of *N. brunnea.*

Values are expressed mean ± SE.

The results of the inhibitory effect of fish extracts on carrageenan induced rat paw oedema are shown in Figure 7. The crude petroleum ether inhibited the oedema volume of 51% with a mean oedema volume of 3.465±0.022 at 4 h, where as the crude ether extract produced to the extent of 56% inhibition of oedema volume with a mean 3.363±0.023 at 4 h. The carrageenan control induced inflammation with a mean oedema volume from 2.500±0.026 at 0 h to 4.000±0.026 at 4 h. The standard drug (reference), diclofenac sodium showed inhibition of oedema volume of 84%.

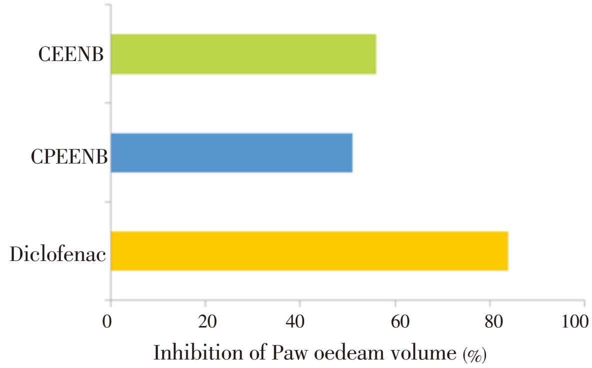

Figure 7. Anti-inflammatory activity of the crude petroleum ether and ether extract of *N. brunnea*.

4. Discussion

In the present study, we assessed the effects of the crude petroleum ether and ether extract of *N. brunnea* against the animal models to establish the analgesic and anti-inflammatory effects. The experimental models used for the screening of fish extracts for its analgesic effect are centrally mediated analgesic activity and it is widely used for analgesic screening based on the enhancement of pain threshold by the compounds present in the crude extracts. In the present study the petroleum ether and ether extract of *N. brunnea* were found to be effective as an analgesic since they significantly (P<0.05) enhance the basal reaction time. The standard drug (reference) buprenorphine (a lipophilic opioid receptor agonist) was used in the present study exerted a significant analgesic effect in all pain models by preventing the sensitization of the opioid receptors. The crude extracts of *N. brunnea* contained compounds like 3, 5- dihydroxy phenyl acetic acid, N-methyl 2, 3- dihydro 3-but-2-enyl indole 5-sulphonic acid and 3-but-2 enyl-indole-5- sulphonic acid. These compounds are capable of relieving pain by preventing the sensitization of the receptors through chemical stimulation there by enhanced the pain threshold[9-10].

The petroleum ether and ether extracts of *N. brunnea* were of considerable biological potential to inhibit the inflammation significantly. The development of oedema induced by carrageenan corresponded to the events in the acute phase of inflammation mediated by histamine, serotonin, bradykinin and prostaglandin produced under an effect of cyclooxygenase in 3 distinct phases. Histamine and serotonin are released in the first phase, kinin may play a role in the second phase and a more pronounced phase is related to the release of the most important mediator prostaglandins in the third phase[11-12]. The standard drug used in the present study are the most widely used drug for the treatment of inflammatory conditions and are non-selective direct inhibitors of cyclooxygenase[13-14]. Our result shows that both the extracts caused a significant inhibition in rat paw oedema only during the 3 and 4 h, where as the inhibition caused at the end of 1 and 2 h was not significant, suggesting the probable mechanism of anti-inflammatory action may be due to the inhibition of prostaglandin biosynthesis by interfering the cyclooxygenase pathway by the combined effect of bioactive compounds present in the crude extracts.

In Conclusion, this study confirmed the analgesic and anti-inflammatory properties of *N. brunnea* observed during the ethno-pharmacological survey. In order to go towards a valuation of this traditional knowledge, further studies like purification, isolation and NMR must be carried out to determine which of these compounds are actually responsible for such properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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