Pharmacological Indications and Uses of Preparations from Dioscorea hispida

The present invention, comprised of the crude aqueous extract of Dioscorea hispida, an indigenous plant from the Philippines, has been shown to have sedative, anti-inflammatory, and analgesic effects. The sedative effect is comparable to sodium barbiturate, a potent sedative and anaesthetic, with D. hispida having a faster onset of lethargy. Moreover, significant anti-inflammatory and analgesic effects are comparable to indomethacin and aspirin. As such, this technology may be applied for general or localized anaesthesia, sleeping pills, pain killers, and modulation of inflammation.
Pharmacological Indications and Uses of Preparations from Dioscorea hispida

Technical Field

This present invention relates to the field of medicinal composition and their uses. More particularly, it relates to the sedative and anti-inflammatory effects of the crude aqueous extract of Nam i (Dioscorea hispida) and its applications.

Background of the Invention

The genus Dioscorea is composed of more than 600 species of flowering plants that thrive in tropical and temperate zones. Some, called yams, have tubers that have been traditionally use as food or an ingredient (Majumdar and Datta 2009). Many have been used for medicinal means such as D. opposita as part of an anti-fatigue mixture (Sun et al. 2012), D. zingiberensis as anti-tumor (WO2012035904), and D. esculenta as an anti-aging agent (WO2012035904).

Dioscorea hispida Dennst. (also known as nami, bagai, kurot, and kayos) is a wild species of yam commonly found growing throughout settled areas in the Philippine archipelago. The tubers are generally round in shape with yellowish sap and flesh. The mature tuber can weigh as much as 50 kilograms (Quisumbing, 1978). While rarely cultivated, it is a staple food in some parts of the Philippines such as Palawan, Cavite, and Batangas. Like other members of the Dioscoraceae family, D. hispida has been reported in folklore for its poisonous and medicinal applications. The tuber contains a water soluble toxicant, dioscorine, which is reported to have insecticidal and antifeedant activities (Banaag et al. 1997; Nagata et al. 1999). The tuber, raw or cooked, is also used as anodyne and maturative in cases of tumor and buboes ( ), and also against arthritic and rheumatic pains and similar affliction (Burkill 1935). At present, there is almost no direct patented application for D. hispida. One patented indication for D. hispida is for the treatment of gonorrhea, and it is prepared as a mixture with other herbs (CN200810130234A).

Prior art on sedatives

Induced sedation and anaesthesia is important in the field of medicine, particularly during surgical, dental, and other invasive medical procedures. At current, anaesthetic agents are mainly made from semi-synthetic compounds. The clinical use is limited to surgical and other invasive medical procedures, with undesired side
effects such as nausea and vomiting, sore throat, muscle aches, confusion, itching, and other significant experiences (Forman 2010; Moore and Hersh 2010) In addition, some important anaesthetics and sedatives such as ketamine and barbiturates are strictly regulated due to drug abuse (Griffiths and Johnson 2005; Wolf and Winstock 2006).

Besides surgery, sedatives are also being used as relaxants, tranquilizers, and sleeping agents, due to the strict regulation and relatively high cost of sedatives, it is imperative that alternative agents of sedation and anaesthesia be explored.

In the Philippines, folk stories in rural areas claimed that eating raw or improperly cooked tuber can result to a long deep sleep. Incidences of death from eating improperly cooked yam have also been reported. Little is known regarding the mechanism of the toxic action, although some claim that it is probably affects the central nervous system (Nagata, 1999). Though there is no definite candidate yet for its intoxicating effect, several molecular candidates may be presented, such as the alkaloid dioscorine (C13H19O2N), methyl protocatechuic acid (C9H8O4 ), caffeic acid (C9H8O4), chlorogenic acid (C16H18O9), p-hydroxybenzaldehyde (C7H6O2), and others.

**Prior art on anti-inflammatory agents**

Anti-inflammatory agents are very commonly used for various indications such as post-op surgical pain, rheumatism, dysmennorhea, muscle pain, headache, chronic inflammation, among others. The commonly prescribed drugs include steroids and NSAIDs (aspirin, diclofenac sodium, ibuprofen, indomethacin, ketoprofen, ketorolac tromethamine, mefenamic acid, naproxen sodium, COX2 inhibitors). However, such drugs are associated with side effects. Steroids can cause systemic side effects that can be life threatening while the other drugs, such as aspirin, may cause gastro-intestinal problems including ulcers, as well as kidney problems (Falk et al. 2008; Khan and McLean 2007).

Ethanolic extracts (90% ethanol by soxhlet's extraction for 48hrs at 55°C) from *D. hispida* has been previously found to have an anti-inflammatory and analgesic effect (Panduranga Murthy et al. 2011). However, there has been no study as to the aqueous extract, which has been found by this invention to be more potent. Recent studies showed that among the candidate anti-inflammatory molecules that possess anti-inflammatory properties include dioscorin, diosgenin, sapogenins, diocoreanone, dioscorea polysaccharide (DPS), phenolics (such as caffeic acid, chlorogenic acid,

5 Summary of the Invention

The object of the invention is to provide means to induce sedation, alleviate inflammation, or relieve pain using a novel extract from Dioscorea hispida or any derivative thereof. With various sedatives such as certain barbiturates associated with adverse drug reaction and being used for drug abuse, and the complications associated with existing anti-inflammatory drugs, extracts from D. hispida can be candidate alternatives to such drugs. This invention aims to be a potent but cheaper alternative to existing sedatives, anti-inflammatory drugs, and pain relievers.

The invention is construed to be used for the following conditions: surgical anaesthesia, treatment for insomnia, tranquilizer, veterinary use, post-op surgical pain, rheumatism, dysmenorrhea, muscle pain, headache, chronic pain (such as in cancer), trauma, auto-immune diseases, hypersensitivity reactions, and chronic inflammation.

Description of Figures and Tables

Table 1 lists the phytochemical composition of the aqueous and ethanolic extracts of D. hispida used in the study. Figure 1 compares the duration of action of the aqueous and ethanolic extracts of D. hispida, while Figure 2 demonstrates the mean onset of lethargy with the extracts. Figure 3 compares the relative anti-inflammatory effect of the aqueous extract to known non-steroidal anti-inflammatory drugs in terms of magnitude of effect and the temporal progression of such effect. Figure 4 shows the magnitude and the temporal progression of the anti-inflammatory effect of the ethanolic extract.

Detailed Description of the Invention

The present invention involves preparations of D. histida extracts, the methods of preparation such, as well as the uses of the preparations.

Sedative Effects of D. hispida

To demonstrate the sedative effect of D. hispida, 3 groups of Swiss Webster mice (n=5 per group) were injected with about 1 mg/kg of D. hispida aqueous extract
(DAE), sodium barbiturate (SB, positive control) and distilled water (DW, negative control). The mice were given from an equivalent of 184.36 mg/kg to 736 mg/kg body weight of *D. hispida* aqueous extracts, or 550 to 1,750 mg/kg of reconstituted lyophilized aqueous extract without mortality. Recently, using a fraction purified by high performance liquid chromatography, only 40 mg per kg body weight was required to get similar sedative effects. The mice were observed for conditions associated with the following sedative effects: period of lethargy, characterized by sluggishness, restive or unruffled attitude; and abnormal drowsiness with or without sleep. The sedative effect of DAE has been found to be significantly higher than distilled water (p<0.01), and is comparable to sodium barbiturate (Figure 1).

Palpebral ptotic response was observed in DAE- and SB-treated mice, and paralysis of the limbs in most test mice, but not in the DW mice. Comparison of the onset of lethargy shows that DAE exhibited a faster onset of action (about 5.8 min) compared with SB or ethanol (p<0.005) (Figure 2).

It appears that DAE is a potent sedative because of its faster onset of action and its longer duration of effect.

**Anti-inflammatory and analgesic effects of D. hispida**

We also demonstrated the anti-inflammatory property of *D. hispida*. Using mouse paw edema test, which is done by injecting 1% carrageenan to a hind paw and observed for 4-5 hours for any changes in the size of the edema, results from DAE show that at a dosage of 0.36 mg per g body weight, both the aqueous extract (DAE) (Figures 3) and ethanol extract (DEE) (Figures 4) inhibited inflammation by 12.86% and 18.39%, respectively (using paw thickness), and 20.69% and 20.86%, respectively (using paw volume).

We also demonstrated the anti-analgesic (painkilling) effect of *D. hispida* by using the formalin test. Intraplantar injections of formalin produce a biphasic behavioral reaction. This behavior consists of an initial phase, occurring about 0-5 min after the injection, followed by a quiescent period, until the second phase between the 20th and 25th min. The formalin injection provokes a painful behavior that can be assessed on a four-level scale (0-3) related to posture and/or behavior.

Mice treated with DAE (about 1 mg/kg) exhibited scale level 2 (raised injected paw) compared to scale level 3 (injected paw licked, nibbled and shaken) for known analgesic drugs paracetamol, aspirin and indomethacin (p<0.05). The mean
cumulative time (0.97 min) at which the DAE-treated mice spent biting/licking the paw was significantly lower than the mean cumulative time exhibited by those treated by paracetamol, aspirin and indomethacin (2.59 min, 3.10 min, and 3.21 min, respectively (p<0.005). These results suggest that at the given dose, DAE exhibited significant analgesic effect at the initial 0-5 min phase, and can bring immediate pain relief in about 1 min.

Such analgesic effect was also observed in the ethanolic extract, but to a lesser degree.

Toxicity of D. hispida extracts

The estimated lethal dose 50 (LD50) of D. hispida aqueous extract was estimated using the Organisation of Economic Co-operation and Development guideline for toxicity testing. A dose of 2500 mg minced tuber per kg body weight was estimated during the assay. For the lyophilized aqueous extract, an LD50 of 1,968 mg per kg body weight was estimated.

Preferred embodiment

The aqueous extract of D. hispida is prepared by washing, weighing, peeling, chopping, and homogenizing the tubers of D. hispida. The osterized extracts are then weighed and soaked in distilled water (1:1 w/w) for three days at room temperature. Afterwards, clear liquid extracts are then collected using cheesecloth, filtration and centrifugation. Different concentrations of the crude D. hispida aqueous extracts (DAE) can be prepared, ranging from 250 mg/mL to 4000 mg/mL. Preferably, the extracts can be lyophilized in order to obtain dry residues that can be quantified for weighing and also for longer storage life. Lyophilized extracts can be reconstituted using distilled water or any polar solvent.

For sedation/sleep induction, the dose of aqueous extract can range from 50 to 1,500 mg/kg body weight, preferably from 100 to 500 mg/kg body weight. For inflammation, the dose can range from 50 to 1,500 mg/kg body weight, preferably from 100 to 500 mg/kg body weight.

The ethanolic extract of D. hispida is similarly prepared, but has been soaked in 95% ethanol (1:1 w/w) for 3 days at room temperature.
Phytochemical screening tests showed that DAE prepared in this manner contains glycoside, alkaloids, saponins, proteins and reducing substances but has no tannins, flavonoids and plant acids (Table 1).

5 Alternative embodiments

Preparation can also be done by using other forms of extraction solvents which are alternative to water, for example alcohol and other polar extraction agents.

Beside liquid preparations, the *D. hispida* extract can also be lyophilized and the final product be in powdered form, which can be reconstituted with polar solvents, such as water, saline or buffered solutions.

In another embodiment, the extract can be further purified using available means, such as by chromatography, precipitation or chemical reactions to isolate the bioactive components. The components can be glycoside, alkaloids, saponins, proteins and reducing substances. Such components can be prepared in liquid or solid form for administration.

For the process, the temperature in the extraction can range from 4-60°C but preferably at room temperature. The final *D. hispida* extract can be stored with or without preservatives.

The length of immersion could range from 1 day to 1 week, preferable from 2-3 days.

*D. hispida* preparations may further take several forms, as such can be administered topically (creams, gels, ointments, patches), orally (tablets, capsules, caplets, suspensions, syrups, elixirs), or of any form for use through transmucosally, intramuscularly, intradermally, intravenously, subcutaneously, intrarthrodially, intraspinaly, intravaginally, inhalationally, or rectally (such as, but not limited to, suppositories and aerosols).

In addition to the tubers, other parts of the plants, such as the leaves, stems and roots, is expected to contain the sedative and/or inflammatory substances. However, such parts are expected to contain less of the active materials.
'Citation List

Patent literature


Non-patent literature

7. Jheng YJ, Tsai WY, Chen KH, Lin KW, Chyan CL, Yang CC, Lin KC. Recombinant dioscorins of the yam storage protein expressed in Escherichia


CLAIMS
1. A process for preparing an extract for *D. hispida* comprising the following steps:
   (1) collecting plant parts of *D. hispida*, (2) cutting them into small pieces, (3) immersing them in aqueous liquid, and (4) removal of the solid component to obtain the aqueous extract.

2. The process according to claim 1, wherein the part of *D. hispida* plant used in the preparation is the tuber;

3. The process according to claim 1, wherein the part of *D. hispida* plant used in the preparation is the leaf;

4. The process according to claim 1, wherein the ratio of the liquid to the plant part is 1:1 wt/wt;

5. The process according to claim 1, wherein the duration of immersion in aqueous liquid is three days or more;

6. A process for preparing an extract for *D. hispida* comprising the steps according to claim 1 with subsequent removal, in part or *in toto*, of aqueous solvent;

7. The process according to claim 6, wherein the removal of aqueous solvent is selected from lyophilisation, desiccation and spray-drying, or any combination thereof;

8. An extract of *D. hispida* obtained by the process according to claim 1;

9. An aqueous composistion of *D. hispida* obtained by the process according to claim 6;

10. A powdered extract of *D. hispida* obtained by the process according to claim 6;

11. A composition containing extracts according to claims 8-10;

12. A reconstituted composition comprising a mixture of the powdered extract according to claim 10;

13. The reconstituted composition according to claim 10, wherein the concentration of the *D. hispida* extract ranges from 50 to 5000 mg per ml of aqueous solvent;

14. Use of reconstituted composition according to claim 11 for sedation of vertebrates;

15. Use of reconstituted composition according to claim 12 for sedation of vertebrates;

16. The use of reconstituted composition according to claim 15, wherein 100 to 500 mg/kg body weight of said extract is administered orally for anti-inflammatory effects in vertebrates;
17. The reconstituted composition according to claim 12, wherein said extract is administered parenterally for sedation of vertebrates;

18. The reconstituted composition according to claim 12, wherein said extract is administered orally for anti-inflammatory effects in vertebrates;

19. The reconstituted composition according to claim 12, wherein extract is administered parenterally for anti-inflammatory effects in vertebrates.

20. A process of purification of the extract according to claim 1, with subsequent performance of high-performance liquid chromatography;

21. The composition obtained using the process according to claim 20;

22. Use of the composition according to claim 21 for sedation of vertebrates;

23. Use of the composition according to claim 21 as anti-inflammatory for vertebrates;

24. A process of purification of the extract according to claim 6, with subsequent performance of high-performance liquid chromatography;

25. The composition obtained using the process according to claim 24;

26. Use of the composition according to claim 25 for sedation of vertebrates;

27. Use of the composition according to claim 25 as anti-inflammatory for vertebrates.
Table 1. Phytochemical screening of *D. hispida* aqueous extract (DAE) and *D. hispida* ethanolic extract (DEE)

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<td>6.06 (acidic)</td>
</tr>
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<td>For tannins</td>
<td>(-)</td>
<td>(+)</td>
</tr>
<tr>
<td>For glycosides</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>For reducing sugars</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>Mayer’s reagent</td>
<td>(+) alkaloids</td>
<td>(+)</td>
</tr>
<tr>
<td>Valsal’s reagent</td>
<td>(+) alkaloids</td>
<td>(+)</td>
</tr>
<tr>
<td>Wagner’s reagent</td>
<td>(+) alkaloids</td>
<td>(-)</td>
</tr>
<tr>
<td>Dragendorff’s reagent</td>
<td>(+) alkaloids</td>
<td>(-)</td>
</tr>
<tr>
<td>Hager’s reagent</td>
<td>(+) alkaloids</td>
<td>(-)</td>
</tr>
<tr>
<td>For sapponins</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>For plant acids</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>For flavonoids</td>
<td>(-)</td>
<td>(-)</td>
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</tbody>
</table>

(-) sign confirms the absence of the component tested
(+) sign confirms the presence of the component tested

Figure 1. Mean duration (hrs) of the sedative effects of crude *D. hispida* aqueous extract (DAE) on the test mice. There was a significant increase in the mean duration of decreased arousability in mice treated with 1 mg/kg *D. hispida* aqueous extract (DAE) compared with distilled water (DW)(p<0.01), and is equivalent to sodium barbiturate (SB).
Figure 2. Mean onset of lethargy of DAE treatment in mice. Injection of 1 mg/kg of the aqueous extract of D. hispida (DAE) induced a faster onset of lethargy on mice compared to sodium butyrate (SB) (p<0.005) and even ethanol. The effect of the ethanolic extract (DEE) is not conclusive, and could be due to ethanol solvent (ETOH).
Figure 3. Anti-inflammatory effect of crude DAE by measurement of the reduction in carrageenan-induced inflammation: (A) mean paw thickness with time; and (B) percentage inhibition of paw thickness increase after 4 hours. Mean inflammatory inhibition of the aqueous extract of *D. hispida* (DAE) is demonstrated by a significant reduction in paw thickness compared with negative controls (distilled water or ethanol, p<0.01). Such effect is comparable to known anti-inflammatory agents indomethacin and aspirin with approximately 10-13% reduction in paw thickness.
Figure 4. Anti-inflammatory effect of ethanolic extract of *D. hispida* crude (DEE) by measurement of the reduction in carrageenan-induced inflammation: (A) mean paw volume with time and (B) percentage inhibition of paw volume after 4 hours. Mean inflammatory inhibition of the aqueous extract of *D. hispida* (DAE) is demonstrated by a significant reduction in paw volume compared with negative controls (distilled water or ethanol, p<0.01). Such effect is comparable to known anti-inflammatory agents indomethacin and aspirin with 20.86% reduction in paw volume.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K36/8945 A61P25/20 A61P29/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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| X        | SEULAH KIM ET AL: "Immunomodulatory effects of Dioscoreae Rhizome Agai nst
Inflammation through Suppressed Production of Cytokines Via Inhibition of the NF- [kappa] B Pathway", IMMUNE NETWORK, vol. 12, no. 5, 1 October 2012 (2012-10-01), pages 181-188, XP055115959 | 1, 2, 8-12, 16, 18, 19 |
| Y        | the whole document                                                               | 23, 27                |

Further documents are listed in the continuation of Box C. [ ]

See patent family annex.

* Special categories of cited documents:
  * A* document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search: 5 May 2014

Date of mailing of the international search report: 23/05/2014

Name and mailing address of the ISA:
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Fax: (+31-70) 340-3016

Authorized officer: Thalmai R., M. chael a
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