Aphrodisiac and Diuretic Activity of Philippine Wild Higher Basidiomycetes, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst., Extract in Male Mice (*Mus musculus*)

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ABSTRACT

Based on ethnomyological studies, the aphrodisiac and diuretic effect of lyophilized extract of Philippine wild medicinal mushroom, *Ganoderma lucidum*, in orally administered male mice was studied in this present work. Mice treated with 250 mg/kg b.w. dose significantly showed the shortest mounting latency (56.00 ± 7.55 sec), intromission latency (41.00 ± 4.00 sec), ejaculation latency (445.67 ± 57.07 sec), and post ejaculatory pause (413.33 ± 15.31 sec) and recorded the highest number of mounts (9.00 ± 1.00) and intromissions (52.67 ± 4.04). The number of pups and the sex ratio of the offspring were also influenced by the extract. Moreover, the extract enhanced the urinary excretion that appeared to vary with dose. Extract-treated mice (5.19 ± 0.32 ml in 100 mg/kg b.w. dose and 7.01 ± 0.19 ml in 250 mg/kg b.w. dose) had higher urine volume when compared to the control (4.77 ± 0.24 ml) and displayed higher diuretic index (1.09 in 100 mg/kg b.w. dose and 1.47 in 250 mg/kg b.w. dose). pH and specific gravity of the urine samples were found higher in treated mice. This first study proved that *G. lucidum* extract has promising potential as effective stimulator of sexual activity with an influence on sex ratio favoring males and of diuretic activity in male mice.

KEYWORDS: *G. lucidum*, Aphrodisiac, Diuretic, Mushrooms

INTRODUCTION

Over the years, medicinal mushrooms have been used as valuable natural resource of bioactive compounds and have been targeted in nutraceutical and pharmacological studies [1]. They contain biologically active metabolites including polysaccharides, proteins, dietary fibers, lipids, lectins, lactones, terpenoids, alkaloids, sterols and phenolic substances [2,3,33], isolated from fruiting body and their cultured mycelia. Some Philippine wild and exotic mushrooms have been studied in a number of functional and/or biologic activities[37]. For instance, antihypertensive effects of *Lentinus sajor-caju*, *Schizophyllum commune*, *Lentinus tigrinus*,...
Ganoderma lucidum, Pleurotus florida, and Collybia reinakeana [4, 5], anti-diabetic and antibacterial activity of L. tigrinus [6], antioxidant properties of Panaeolus antillarum, L. tigrinus, L. sajor-caju [7, 8], and embryotoxic and teratogenic effects of G. lucidum, L. sajor-caju and Pleurotus ostreatus [9, 10].

Ganoderma lucidum (W.Curt.:Fr.) P. Karst., is a red varnished, soft (when fresh), corky, and flat basidiomycetous mushroom that commonly found growing on logs and woods in the forest. This wild mushroom has been utilized by Filipinos because of so many healthful benefits. Its dried fruiting body has been prepared and consumed as tea and commonly used to treat or prevent diabetes, hypertension, stress, and arthritis. In recent years, G. lucidum has been intensively studied because of its valuable chemical components and various biological activities particularly in immunomodulating and anticancer properties [1]. With this regard, many Ganoderma-based products such as coffee, soap, lotion and dietary supplements are already available in the market.

Aphrodisiac (derived from Aphroditē, the Greek goddess of love) is any significant agents (food, drinks or drug) that enhances the sexual behavior, induces fertility, and increases pleasure and performance. Malviya et al. [11] categorized aphrodisiac into three groups according to their mode of action: substances that increase libido (i.e., sexual desire, arousal), substances that increase sexual potency (i.e., effectiveness of erection) and substances that increase sexual pleasure. Many herbal plants have been widely studied for their aphrodisiac activity. Some plants that exhibit this important property include Tribulus terrestris, Withania somnifera, Eurycoma longifolia, Avena sativa, Ginkgo biloba, and Psoralea coryifolia [11]. Other organisms such as Lytta vesicatoria, Pseudepelatum olacoides, Crocus sativus, Bufo marinus, Myristica fragrans, Theobroma cacao and other plants have been investigated for their aphrodisiac activity in-vivo and in-vitro [12,32].

Diuretic is any substance that promotes the formation of urine, increases sodium excretion and adjusts the volume and body fluids composition. This agent is advantageous to many physiologic-associated diseases such as hypertension, congestive cardiac failure, ascites and pulmonary diseases [13]. Some medicinal plants mentioned in Ayurvedic system of medicine that exhibit diuretic properties includes Abelmoschus esculentus (Bhindaka), Bacopa monnieri (Brahmi), Barbarea vulgaris (Cress), Boerhavia diffusa (Punarnava), Emblica officinalis (Amla) [14].

Accordingly, most of the literatures and reports on aphrodisiac and diuretic activity dealt on the utilization of medicinal and/or herbal plants, but no previous work found on the use of mushrooms. Moreover, this study was interestingly carried out because of the ethno-mycological knowledge that G. lucidum is used to improve sexual performance and boost urine production. Therefore, this first study demonstrated that G. lucidum extract exhibits aphrodisiac and diuretic activity in male mice.

MATERIALS AND METHODS

Source of mushroom:

Fruiting bodies of G. lucidum grown on rice straw-sawdust based formulation (7:3 ratio) were obtained from the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines. The fructing bodies were air-dried for 3-5 days prior to extraction.

Extraction of bioactive components:

Air-dried mushroom sample (20 g) was pulverized using a blender and extracted in 600 mL hot water at 80-90°C in a water bath for 2 hours. The milled mushrooms were separated from the extract by filtration using filter paper No. 2 then filter sterilized through 0.45 µ filters. The filtrate was freeze-dried for 3 days to obtain the lyophilized form of extract. The extract was used for aphrodisiac and diuretic evaluation.

Experimental animals:

Healthy adult (8-week old) male and female mice weighing 25-33 g were used in this study. Mice were housed individually in cages, fed with standard rat pellet diet and water ad libitum, maintained under standard conditions (12 h light and 12 h dark regime; 25°C). They were acclimatized in this condition for 7 days prior to evaluation.

Aphrodisiac activity evaluation:

Sexually inactive male mice were screened and selected based on the parameters established by Burses et al. [15]. Selected mice were randomly divided into three groups of three mice in each group. Groups I and II mice were orally administered with G. lucidum lyophilized extract at 100 mg/kg and 250 mg/kg b.w., respectively, in single dose regimen; and group III non-treated mice served as control. After 2 hours of treatment administration, male mice were then introduced to the receptive female mice to assess their sexual behaviour in 30 minutes. The different parameters evaluated include; latency of first mount, number of mounts, latency of first intromission (characterized by pelvic thrusting and springing dismount), number of intromissions, latency of ejaculation (characterized by longer, deeper pelvic thrusting and slow dismount followed by a period of inactivity) and post ejaculatory pause (time from ejaculation to next mount). Mice were watched for pregnancy
and birth of offspring. The number of pups of each mouse and the number of male and female pups were recorded [31].

**Diuretic activity screening:**

A method used in screening diuretic activity of *G. lucidum* extract was followed after Kau et al. [16] with modifications. Mice were randomly divided into three groups of three animals each as follows: Groups I (100 mg/kg b.w.) and II (250 mg/kg b.w.) mice were orally administered with *G. lucidum* lyophilized extract and group III non-treated mice. Immediately after administration, the mice were individually placed in their cages. Urine was collected and measured in a graduated cylinder after 5 hours. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g b.w. The protein, glucose, pH, and specific gravity of the urine samples were analyzed at the end of experimental period.

**Statistical analysis:**

The statistical analysis of data was carried out using one way ANOVA and treatment means were compared at 5% level of significance in LSD using SAS statistical program.

**RESULTS AND DISCUSSION**

**Aphrodisiac activity:**

Aphrodisiac substances are used to improve sexual behaviour and satisfaction and to treat sexual dysfunction in animals and humans. Available synthetic drugs like sildenafil citrate (viagra) have limited efficacy and uncertain safety because of various side effects such as headache, flushing, dyspepsia and nasal congestion [17]. Therefore, it is an interest of the present work to search for a natural, effective, and safe source of substances, aside from plants, with promising aphrodisiac activity. The effect of *G. lucidum* extract on the sexual behaviour of male mice in single dose regimen is presented in Table 1. It was observed that the sexual behaviour activity was increased in mice administered with *G. lucidum* extract. Administration of 250 mg/kg b.w. dose significantly showed the shortest mounting latency (56.00 ± 7.55 sec), intromission latency (41.00 ± 4.00 sec), ejaculation latency (445.67 ± 57.07 sec), and post ejaculatory pause (413.33 ± 15.31 sec) and recorded the highest number of mounts (9.00 ± 1.00) and intromissions (52.67 ± 4.04). However, treated animals with 100 mg/kg b.w. dose registered lower sexual behaviour activity when compared to 250 mg/kg b.w. dose-treated mice but significantly higher than those in control.

**Table 1:** Effect of single dose oral administration of *G. lucidum* extract on the sexual behaviour of male mice.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G. lucidum extract-treated mice</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 mg/kg b.w.</td>
<td>250 mg/kg b.w.</td>
</tr>
<tr>
<td>Mount latency (sec)</td>
<td>112.33 ± 13.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.00 ± 7.55&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of mounts</td>
<td>5.00 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.00 ± 1.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intromission latency (sec)</td>
<td>60.00 ± 12.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>41.00 ± 4.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>No. of intromissions</td>
<td>51.33 ± 5.51&lt;sup&gt;c&lt;/sup&gt;</td>
<td>52.67 ± 4.04&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ejaculation latency (sec)</td>
<td>631.00 ± 55.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>445.67 ± 57.07&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post ejaculatory pause (sec)</td>
<td>444.00 ± 30.81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>413.33 ± 15.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM, and means in each parameter with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD.

Since all the females inseminated by the extract-treated and control males became pregnant, the number of pups and sex ratio of the offspring were also determined (Table 2). Interestingly, there was a significant increase in the number of pups fathered by the 250 mg/kg b.w. extract-treated males. It is also noteworthy that the two extract dosages altered sex ratio favoring male offspring. Apparently, *G. lucidum* lyophilized extract could potentiate aphrodisiac activity which strongly indicated by the increased sexual behaviour in male mice with an influence on the number of pups and their sex ratio. This conform with the previous work of Subramoniam *et al*. [18] who evaluated the aphrodisiac activity of the elephant creeper (*Argyria nervosa*) which showed a promising potential in stimulating male sexual behaviour that affects the male/female ratio of the offspring. Since this present study is a preliminary work on the utilization of mushroom in aphrodisiac activity evaluation, the mechanism in improving the sexual activity in male mice by *G. lucidum* extract could not be explained at this time. Therefore, it is necessary to explore the possible mechanisms of actions in the next study.

**Table 2:** Effect of *G. lucidum* extract on the number of pups and sex ratio of offspring fathered by the treated males.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>G. lucidum extract-treated mice</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100 mg/kg b.w.</td>
<td>250 mg/kg b.w.</td>
</tr>
<tr>
<td>No. of pups</td>
<td>4.67 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.33 ± 1.15&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sex ratio of pups (male/female)</td>
<td>1.50 ± 1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.67 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM, and means in each parameter with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD.
Many aphrodisiac evaluations have extensively focused on plants. In ayurvedic system of medicine, the plants with important aphrodisiac property include *Myristica fragrans* (*Myristicaceae*), *Allium tuberosum* (*Zingiberales*), *Crocus sativus* (*Iridaceae*), *Palisota hirsuta* (*Camelinales*), *Mundia whitei*, *Eurycoma longifolia* (*Simarubaceae*), *Lepidium myenii* (*Brassicales*), *Montanoa tomentosa*, *Securidaca longepedunculata* (*Polygalales*), *Durio zibethinus* (*Bombaceae*), *Dactylorhiza hatagirea* (*Orchidales*), *Securidaca longepedunculata* (*Polypogonales*), *Syzygium aromaticum* (*Myrtaceae*), *Vanda tessellata*, and *Butea frondosa* (*Papilionaceae*) [19]. The suspension of date palm (*Phoenix dactylifera*) pollen improved sperm quality and enhance fertility in the male adult rat [20]. Aqueous extract of *Fadogia agrestis* stem significantly increased the mount and intromission frequency, reduced mount and intromission latency, increased the blood testosterone concentrations, which may be responsible for the aphrodisiac effects and various masculine behaviours in male albino rats [21]. In chronic administration of aqueous extracts of *Hybanthus enneaspermus* increased the number of mounts, ejaculations, and intromissions and elevated the testosterone levels in sexually inactive male rats [22].

Subramoniam *et al.* [18] explained that sexual behaviors could be enhanced by elevated testosterone levels, changes in neurotransmitter levels, actions in the cells or alterations in the signaling system such as nitric oxide signalling. This improved sexual behaviour could be attributed to the bioactive components of the aphrodisiac plants. *Eurycoma longifolia* enhanced the sexual qualities of the middle aged male rats, and the active constituents of this plant are alkaloids, lactones and phenolics [23]. Estradiol and flavonoid extracted from date palm have positive effects on the sperm quality [20]. Moreover, the chemical composition of *Chline venosa* has revealed the presence of acetophenone-2-O-[β-D-apiofuranosyl-(1',6')-β-D-glucopyranoside] and acetophenone-2-O-[β-D-glucopyranoside], along with five known compounds, α-morroniside, sweroside, dierdrose, daucosterol and β-sitosterol which contribute to the aphrodisiac potential of this plant [24]. Similarly, *G. lucidum* also contains a wide variety of bioactive compounds that exhibit different functional activities. The bioactive molecules of this mushroom are mostly described to be polysaccharide (β-glucan) and triterpene (ganoderic acids) fractions [25, 26]. These substances present in *G. lucidum* could possibly the potent stimulators of sexual activity in male mice. However, the specific constituents involved in aphrodisiac activity of *G. lucidum* must be isolated and identified [34, 35].

**Diuretic activity:**

Diuretics may be very useful in many health conditions. Therefore, the increasing search for medicinal agents with diuretic potentials has necessitated the need for screening medicinal resource. In this study, we screened the diuretic potential of *G. lucidum* in male mice. The extract enhanced the urinary excretion that appeared to vary with dose (Table 3). The cumulative urine volume of extract-treated mice (5.19 ± 0.32 ml in 100 mg/kg b.w. dose and 7.01 ± 0.19 ml in 250 mg/kg b.w. dose) was significantly higher compared to the control (4.77 ± 0.24 ml). In view of that, mice given with 250 mg/kg b.w. dose displayed higher diuretic index of 1.47 than 100 mg/kg b.w. dose with 1.09. These results therefore dictate that the chemical ingredients from *G. lucidum* obtained through hot water extraction have promising diuretic potential in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cumulative urine volume (ml / 100 g b.w. / 5 hr)</th>
<th>Diuretic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.77 ± 0.24a</td>
<td>---</td>
</tr>
<tr>
<td>100 mg/kg b.w. dose</td>
<td>5.19 ± 0.32b</td>
<td>1.09</td>
</tr>
<tr>
<td>250 mg/kg b.w. dose</td>
<td>7.01 ± 0.19c</td>
<td>1.47</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM, and with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD. Diuretic index = volume treated group / volume control group.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein</th>
<th>Glucose</th>
<th>pH</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>± trace</td>
<td>negative</td>
<td>6.50 ± 0.50a</td>
<td>1.010 ± 0.01c</td>
</tr>
<tr>
<td>100 mg/kg b.w. dose</td>
<td>± trace</td>
<td>negative</td>
<td>6.67 ± 0.29b</td>
<td>1.010 ± 0.00c</td>
</tr>
<tr>
<td>250 mg/kg b.w. dose</td>
<td>± trace</td>
<td>negative</td>
<td>6.83 ± 0.29c</td>
<td>1.015 ± 0.00d</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM, and with the same letter of superscript are not significantly different from each other at 5% level of significance using LSD.

The proteins, glucose, pH, and specific gravity of urine samples collected over the five hours were analyzed and the results are depicted in Table 4. No apparent difference was observed between controls and treated with the two dosages. Proteins and glucose were found traceable and negative, respectively. On the other hand, higher urine pH (6.83 ± 0.29) and specific gravity (1.015 ± 0.00) were recorded in 250 mg/kg b.w. treated mice than with lower dose and control. This significant increased in pH values reinforce the notion that carbonic anhydrase inhibition is one of the possible diuretic mechanisms of action.
Recently, the diuretic potential of three plants from Menispermaceae family was investigated by Hullatti et al. [13]. They found out that at 100mg/kg both Cyclea peltata and Stephania japonica root extracts significantly increased the urinary output. However, at 200 mg/kg dose all the three extracts (including Cissampelos pareira) significantly increased urine volume and urinary electrolytes, comparable to that of furosemide. In addition, both aqueous and ethanolic extracts of Lawsonia inermis leaves showed diuretic effect in a dose dependent manner which are indicated by increase in both water and excretion of sodium, potassium and chloride. This activity was associated to the phytochemicals of Lawsonia inermis leaves such as alkaloids, tannins, phenolic compounds, triterpenoids, and especially flavonoids [27]. Previous works demonstrated that there are various bioactive compounds from plants responsible for diuretic activity including flavonoids, saponins or organic acids [28].

Generally, mushrooms are rich source of various bioactive compounds. These include alkaloids, flavonoids, fixed oil, glycosides, phenols, resins, saponins, steroids, tannins, and terpenoids [29]. The other biologically active compounds, such as polysaccharides, triterpenes, proteins, and lipids, were isolated from G. lucidum [30]. The diuretic effect exhibited by G. lucidum could be accounted to its wide variety of biologically active substances, thus, compounds actively involved in this activity must be isolated and identified. This valuable effect may be due to the stimulation of regional blood flow or inhibition of vasopressin or by producing inhibition of tubular reabsorption of water and anions, that probably caused by active components of G. lucidum.

**Conclusion:**

Collectively, lyophilized extract of G. lucidum exhibited significant aphrodisiac and diuretic activity in male mice. The extract stimulated the sexual behaviors of male mice and influenced the number of pups and sex ratio of the offspring. Moreover, extract is also an effective diuretic agent as it significantly increased the urine volume and improved the diuretic index. With important findings, it is necessary to investigate the possible mechanism of actions of the extract and elucidate the bioactive constituents responsible for these important functional activities in future studies.

**REFERENCES**


