Biological activity of the Tiger mushroom (Lentinus tigrinus) with notes on its assessment for therapeutic consideration

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ABSTRACT

This study has been initiated to explore the therapeutic potential of L. tigrinus. Evaluation of lethal effects and responses of mice to Lentinus tigrinus were undertaken. Intravenous lethal dose 50 (LD₅₀), safe intravenous dose and other safety indices were also determined to validate its prospective therapeutic value. The experiment used 30 male BALB/c mice randomly distributed into 6 treatments. Each mouse received 200 µl of each dose of L. tigrinus extract (LTE) (3 mg/mouse, 7.50 mg/mouse, 15 mg/mouse, 22.50 mg/mouse, 30 mg/mouse). Observation for mortality, perceptible responses such as piloerection, eye secretions, weakness and anorexia were carried out at 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 36 h and 48 h post-treatment with LTE wherein median lethal dose, safe IV dose, safety indices and dose-related responses were based from. Results showed that treatment with higher LTE concentrations (15 - 30 mg/mouse) elicited perceptible responses earlier preceding death of significantly higher number of mice compared to delayed onset of perceptible responses with no associated mortality in treatments with lower doses. Data also identified the threshold dose at 3 mg/mouse which triggered observable responses of mice while concentrations below the threshold dose have neither effect on perceptible responses nor on death in mice and linked estimated safe IV dose at 7.50 mg/mouse with zero mortality. Identification of the LD₅₀ at 28.47 mg/mouse demonstrated its potent lethal effect in mice. Computed safety indices showing low therapeutic index (1.99), therapeutic ratio (0.67) and a safety factor (0.02) for LTE confirms its low margin of safety when used for therapeutic purposes.

INTRODUCTION

Mushrooms have many important uses to humans. These are used as food supplement in various cultures and are cultivated as a source of income. The high amounts of proteins, vitamins, fats, carbohydrates, amino acids and minerals contained in mushrooms favored its nutriceutical value [1].

In recent times, mushrooms have assumed greater importance in the medical field as a potential remedy for various diseases [7]. Pleurotus tuber-regium is useful in some combinations to cure headache, stomach ailments, colds, fever, asthma and high blood pressure while Lentinus tuber-regium and Lentinus tigrinus (L. tigrinus) are used for the treatment of dysentery and blood cleansing activity, respectively [1].

Many of Philippines’ mycodiversity are still in the wild and remained unstudied. One species of mushroom that needs to be explored is the tiger mushroom (L. tigrinus). Interest on the biologic activity of mushrooms is largely unknown for most information is limited to taxonomical verifications, cultural adaptation, survey analyses of mineral composition and extraction of its active components. Therapeutic claims of wild mushrooms are equivocal and anecdotal based mainly on speculations [11].

Research work has been initiated to explore the therapeutic potential of L. tigrinus. Investigation of animal responses after intravenous (IV) administration of the mushroom was undertaken and relative data on established safe IV doses, lethal dose 50 (LD₅₀), therapeutic index and therapeutic ratio were discussed to authenticate its prospective beneficial effect.

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MATERIALS AND METHODS

Experimental Animals:
Thirty (30) male BALB/c mice, 2 to 4 week old, with average weight of 30 g were used. The mice were randomly distributed in six rectangular cages that measured 15x5x5 inches. Five mice in each treatment were separately placed in a cage pre-fabricated with five small divisions. Mice were kept to acclimatize in a restricted room for one week. Fresh feed pellets and drinking water were given daily. Management was in accordance with the Philippine Animal Welfare Law and Institutional Animal Care and Use Committee Guidelines.

Extraction of L. tigrinus mycelia:
One hundred (100) grams of dried L. tigrinus mycelia was provided by the Center for Tropical Mushroom Research and Development of Central Luzon State University and extracted with hot water following the procedures of Reyes et al. [11]. The samples were cut into small pieces and ground using mortar and pestle. The samples were placed in a flask with 500 ml distilled water and heated for 2 h at 80 to 90°C in a water bath. Extraction was done with slight agitation for 2 h. The sample was filtered (No. 2, Toyo Co., Japan) and frozen at -80°C overnight before freeze drying (Heto Holten Dry Winner 3).

Intravenous (IV) administration of L. tigrinus extract (LTE):
Dried extracts of L. tigrinus were dissolved in sterile water for injection to come up with the different concentrations for treatments (T1, 3 mg/mouse; T2, 7.5 mg/mouse; T3, 15 mg/mouse; T4, 22.5 mg/mouse; T5, 30 mg/mouse; and T6, sterile water for injection as the control). Two hundred (200) μl of each concentration was injected via the tail vein of mice in each treatment using a tuberculin syringe and a 25-gauge needle. Mice were deprived of feeds 3 hrs after the IV injection.

Monitoring of body responses:
Observation of perceptible responses such as changes in hair coat (piloerection), eye secretions, weakness and anorexia were carried out at 30 min, 1 h, 3 h, 6 h, 12 h, 24 h, 36 h and 48 h after treatment with LTE. Weakness was recognized as hypoactivity, inability of mice to ambulate or sluggish movement when stimulated; anorexia was assessed by non-reduction of the weight of feed initially given or no indication of any feed consumption after weighing the actual amount of feed given before and after treatment, including the left over; and quality of feces.

Evaluation of the median lethal dose (LD50):
The number of deaths per treatment was counted within the prescribed time of observation. The LD50 of LTE on experimental subjects was computed using the Probit's method of determining lethal points [5].

Safe intravenous dose, therapeutic index, therapeutic ratio and safety factor:
The safe IV dose was determined between doses where mortality occurred at less than 50%. The highest possible dose where no mortality was recorded was considered as the safe IV dose. Safety measures also covered evaluation of therapeutic index, therapeutic ratio and safety factor for LTE. Therapeutic index (TI) was computed as the ratio of median lethal dose and the median effective dose (TI = LD50/ED50). The therapeutic ratio (TR) was obtained as the ratio of 25% lethal dose and 75% effective dose (TR = LD25/ED75) while the Safety factor (SF) was derived from the ratio between 1% lethal dose over the 99% effective dose (SF= LD1/ED99). LTE concentrations that caused mortality and perceptible changes in the experimental animals were the basis in estimating for the lethal and effective doses of LTE, respectively.

Statistical Analysis:
All data were taken as mean responses of each treatment. Mean responses between treatments were compared by Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Physiological alterations:
Roughness of hair coat or piloerection related to the treatment of LTE was noted as physiological alteration. At 3 h post-treatment with LTE, percentage of mice showing piloerection was significantly higher in the group treated with 30 mg compared to mice given lower levels of LTE (P<0.01). After 6 h post-treatment, percentage of mice exhibiting this response was significantly higher (P< 0.01) in groups that received 30 mg compared to groups of mice that received lower LTE concentration. In addition, the percentage of mice with piloerection were also significantly higher (P<0.01) than number of mice that manifested the response at 3 h. From 12 to 24 h, comparable percentage of mice treated with 30 mg and 22.5 mg exhibited piloerection but the comparable
number of mice showing the response was significantly higher than those that received lower LTE concentrations (P<0.01). Number of mice with the same response were significantly higher than the number of mice observed with piloerection at 3 and 6 h (P<0.01). Significantly higher percentage of mice were seen with the response related to treatment with 15 mg and 7.5 mg compared to those seen with piloerection on the previous 3 and 6 hrs (P<0.01). At 36 h, % of mice with piloerection was significantly higher in a group that received 22.5 mg compared to other treatments (P<0.01) but mice that showed response was comparable with the number of mice which manifested the response in the past 12 and 24 h. Treatment with 15 mg LTE lead to comparable percentage of mice that demonstrated piloerection at 24 h while treatment with 7.5 mg and 3 mg contributed to significantly higher number of mice with piloerection at 36 h compared to the number of mice demonstrating the response in the past 24 h (P<0.01).

Weakness:

Percentage of mice showing weakness, hypo-activity or inability to ambulate post-treatment was also noted. No evidence of weakness was observed in mice from all treatments during the first 3 h. From 6 to 24 h post-treatment with higher dose of LTE (30 mg, T5), significantly higher number of mice demonstrated weakness (P<0.01) compared to mice that received lower levels of LTE treatment. This LTE-evoked response was comparable across time points in mice treated with 30 mg, unlike with the gradual increase in the number of mice showing weakness as a result of treatment with lower dose of LTE (22.5 mg) (P<0.01). At 36 h, higher levels of treatment with LTE contributed to significantly higher percentage of mice that demonstrated weakness compared to lower levels of treatments (P<0.01). Treatments with LTE contributed to significantly higher number of mice manifesting weakness compared to previous time points (P<0.01).

Formation of eye crusts:

Formation of eye crusts in mice post-treatment with LTE is also assessed. No eye crust was demonstrated by mice from all treatments on the first 12 h post-treatment with LTE. It was at 24-h post treatment when significantly higher percentage of mice from the group treated with 30 mg LTE exhibited eye crusts compared to lower number of mice that showed eye crusts after treatment with lower levels of LTE (P<0.01). At 36 h, significantly higher percentage of mice exhibited eye crusts with the treatment of 22.5 mg LTE compared to the number of mice showing responses elicited by lower concentrations (P<0.01). In addition, mice showing formation of eye crusts in each level of treatment were significantly higher than the percentage of mice showing the responses for the past 24 h (P<0.01).

Anorexia and fecal quality:

The percentage of anorexic mice as a response related to the treatment of LTE is also assessed. Data show that none of the mice in all treatments manifested anorexia for the first 24 h. At 48 h, treatment of mice with 22.5 mg LTE produced significantly higher percentage of anorexic mice compared to the number of mice with the anorexic signs that received lower concentrations of LTE (P<0.01). Treatment with LTE did not contribute to any alterations in fecal quality of mice within 48-h post-treatment.

Lethal effect and evaluation of the LD₅₀:

Results showed that no deaths were evident in all treatments at the first 6 h of observations. However as observation time advanced, number of deaths gradually increased in most treatments. At the end of the 48-h post-treatment observation, group of mice that received 30 mg/mouse had significantly higher deaths compared to those treated with lower levels of LTE (P<0.05). The LD₅₀ derived from the mortality data of mice after treatment with LTE was 28.47 mg/mouse (949 mg/kg) (Probit’s data not presented).

Dose-response curves, safe intravenous (IV) dose, therapeutic index (TI), therapeutic ratio (TR) and safety factor (SF):

The dose-response curves of LTE is presented in Figure 1 describing the cumulative response of mice to different doses of LTE plotted against percentage mortality and the perceptible responses described. Based on the results, 3 mg/mouse was established as the threshold dose, a concentration that initially triggers observable or perceptible responses of mice to LTE. In using LTE concentrations below the threshold-dose, no deviation of normal responses and no adverse effects in the population were noted. From the same data, it appeared that the minimum dose where no mortality can be recorded was at 7.50 mg/mouse and this is considered as the safe IV dose of LTE, although a few number of mice demonstrated piloerection at a latter onset. The therapeutic potential of LTE was evaluated and data showed that it has a therapeutic index of 1.99, a therapeutic ratio of 0.67 and a computed SF of 0.02 (Table 1).
Fig. 1: Dose response curves of LTE. Effective curve (blue) illustrates the doses where perceptible responses (piloerection, eye crusts, weakness and anorexia) in mice were observed. Lethal curve (red) represents the dose that induced mortality in the population. The area between the two curves depicts safe doses of LTE.

Table 1: Computed safety indices for LTE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Computed Value</th>
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<tbody>
<tr>
<td>Therapeutic Index</td>
<td>1.99</td>
</tr>
<tr>
<td>Therapeutic Ratio</td>
<td>0.67</td>
</tr>
<tr>
<td>Safety Factor</td>
<td>0.02</td>
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Administration *L. tigrinus* extract (LTE) in mice as experimental animals triggered piloerection, weakness, formation of eye crusts and anorexia with no effect on fecal quality. Results showed that treatment with higher LTE concentrations elicited earlier onset of the perceptible responses preceding the death of a significantly higher number of mice unlike the observed delayed onset of perceptible responses with no mortality associated with the treatment of lower doses. Treatment with LTE may induce sympathetic stimulation of muscles under the skin to contract and effect piloerection. Piloerection has been described as a common external symptom that accompanies many infectious diseases, parasitic worm infections and nutritional disorders that limit copper resources for maintaining healthy hair of primates [10]. Weakness in treated mice was accompanied by muscle twitching and shivering indicative of muscle excitation in response to LTE treatment. It appears that LTE causes excitatory effect on skeletal muscle junctions, caters release of the excitatory neurotransmitter acetylcholine that influences muscle twitching and accumulation of acetylcholine at the neuromuscular junctions that influence muscle twitching or myoclonus as indicated by shivering and weakness in mice. Weakness and inability of mice to ambulate post-treatment with higher doses of LTE follow the observation of Auerbach [3] who reported walking difficulty and dizziness in mice 30 min to 2 h after ingestion of wild mushroom *Amanita pantherina* (panthercap). Marked weakness, disorientation, anxiety, mydriasis, tachycardia and hyper-reflexia reportedly developed in mice 30 min to 4 h after ingestion of hallucinogenic or magic mushrooms like *Psilocybe, Panaeolus, Copelandia, Gymnopilus, Pluteus,* and *Conocybe* [4]. The LTE-evoked weakness, however, does not equate the flaccid type of weakness shown by mice with panthercap poisoning described in the studies of Hallen *et al.* [6]. Formation of crusts in the eyes explains excessive lacrimation due to over-stimulation of acetylcholine in the muscarinic receptor of the eyes of mice treated with LTE. The appearance of exocrine gland secretions such as tears, sweat and saliva in experimental animals injected with wild mushroom *Amanita 30* min to 6 h after treatment have been cited [12]. Anorectic condition in mice after treatment with higher concentrations of LTE can be attributed to the hypo-activity, inability to ambulate and the absence of desire to eat that can alter feeding behavior of mice. Signs of anorexia observed in the study on LTE are similar as the adverse effects of lyophilized extracts of the wild mushrooms *Panaeolus subalteatus, Macrolepiota procera* and *Hygrophoropsis aurantia* claimed by others [2]. Observations showing non-alteration of fecal quality in this experiment explain that IV administration of LTE has no direct effects on the digestive process of mice in contrast to the gastrointestinal irritation described [12] due to a wide variety of undetermined toxins of wild mushrooms.
Mushrooms such as *Agaricus, Boletus, Entoloma, Gomphus, Hebeloma, Russula, Scleroderma* and *Tricholoma* are claimed as gastrointestinal irritants although the toxic principles involved are unknown [4]. Although the toxic principles responsible for the deaths of mice related to treatment with LTE is still unknown, observations noted in this study suggest that the extreme muscle stimulation manifested by shivering and myoclonus leading to spastic paralysis exhibited by each affected mouse lead to death. Mice mortality post-treatment with LTE is associated spastic paralysis brought about by higher concentrations of LTE in contrast to the absence of death in control group. The lethal effect of *L. tigrinus* can be confirmed by its median lethal dose (LD$_{50}$) at 28.47 mg/mouse (949 mg/kg body weight of mice). The computed LD$_{50}$ value of LTE in our study rated *L. tigrinus* slightly more toxic than *Cortinarius speciosissimus* and *Cortinarius orellanus* with LD$_{50}$ values of 2.0 g/kg and 3.2 g/kg, respectively [8] but less toxic compared to the 10 mg lethal dose of *Amanita phalloides* amatoxin reported by Patowary [9]. Rapid induction of death after LTE injection are similar to the lethal effects of wild mushroom *Amanita phalloides* amatoxins and lyophilized extract of *Panaeolus subalveatus* and *Macrolepiota procera* injected intraperitoneally at dose rate 1,000 mg/kg [2,12].

Data on safety indices demonstrates the characteristics of LTE as a substance with a very low therapeutic index (TI), therapeutic ratio (TR) and safety factor (SF). Data explain that LTE with a low TI and TR can presumably be administered with extremely low safety than one with a higher TI or TR because a substance with a higher therapeutic index and therapeutic ratio is much preferable for treatment for the reason that a higher dose is needed to reach a lethal threshold to treat than the dose required to just elicit an effect. Any drug with a therapeutic ratio of 1.99 would require twice as much its effective dose to exert its desired effect. The computed SF of LTE at a value lower than 1.0 likewise connotes that *L. tigrinus* is a potentially toxic substance for intravenous administration in mice. As LTE is a relatively toxic substance having a narrow margin of safety, extreme caution should be taken into consideration if the mushroom has to be used as functional food, natural-based pharmaceutical product or a potential pharmacological substance.

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