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In vitro Doses and Incubations Dependent Thrombolytic Potential Study of Edible Mushrooms *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* Available in Bangladesh

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Authors' contributions

This work was carried out in collaboration between both authors. Authors MMUP designed the study, and wrote the protocol. Author MRI wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The study was carried out to investigate the thrombolytic potential of methanolic extracts of edible mushrooms.
Study Design: Experimental study.
Place and Duration of Study: Department of Biochemistry, Primeasia University, August 2014 to December 2014
Methodology: An *in vitro* thrombolytic model was used to check the thrombolytic effect of *Pleurotus ostreatus, Ganoderma lucidum and Lentinula edodes* along with Streptokinase as a positive control and water as a negative control. In this study two concentrations of the extracts i.e. 400µg/ml and 800 µg/ml were tested at three time intervals, 24 hrs, 48 hrs and 72 hrs duration of incubation at 37ºC for observing maximum clot lysis.
Results: At 400 µg/ml dose, *Pleurotus ostreatus* showed highest clot lysis activity in 72 hrs of incubation, 18.62% while *Ganoderma lucidum*, and *Lentinula edodes* showed 17.01% and 9.02% in the same incubation. Similarly, at 800 µg/ml dose, the thrombolytic activities were 63.35%,

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34.16%, and 29.00% respectively for *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes.* Our study clearly demonstrated that *Pleurotus ostreatus* exhibits maximum 63.37% clot lysis at 800 µg/ml concentration in 72 hrs of incubation with reference to Streptokinase 86.43%. The thrombolytic nature of the mushroom extracts was found to be significant (P<0.0001), when compared with the negative control (water) at different doses. The relative thrombolytic activity of *Pleurotus ostreatus, Ganoderma lucidum and Lentinula edodes* was found to significant at different doses (P<0.0001) and at various incubation periods (P<0.0001).

Conclusion: The result of our findings indicated that percentage of clot lysis is directly proportional to the concentration and incubation time of the extracts.

Keywords: Clot lysis; thrombolytic agents; oyster; shiitake.

1. INTRODUCTION

Thrombosis is the formation of a blood clot within a blood vessel causing a partial or total obstruction, which prevents blood from flowing normally through the circulatory system. It is caused due to imbalance of homeostatic system of the body [1]. Modern pharmaceuticals have developed many drugs over the years with the purpose of dissolving clots, such as alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen [2,3] Among these drugs streptokinase and urokinase are widely used because of the low cost as compared to other thrombolytic drugs [4,5]. But due to the weak substrate specificity of these first generation drugs (streptokinase and urokinase), they lead to systemic fibrinolysis, anaphylactic reaction and bleeding complications [6]. Moreover multiple treatments with streptokinase are restricted in a given patient, as a result of immunogenicity [7]. Because of the shortcomings associated with the thrombolytic drugs, it is necessary to find an attractive alternative and to develop improved recombinant of these drugs [8].

At present there are at least 270 species of mushroom that are known to have various therapeutic properties [9]. Medicinal mushrooms offer potentially important therapeutic properties including antioxidants, anti-hypertensive, cholesterol-lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic, anti-viral and anti-microbial, thrombolytic activity.

Ganoderma lucidum has the longest historical usage for medicinal purposes [10]. This mushroom possesses many different medicinal properties dependent on the stage and environment of its growth [11]. Traditionally, it has been widely used in the treatment of hepatopathy, chronic hepatitis, nephritis, hypertension, arthritis, neurastheine, insomnia, bronchitis, asthma and gastric ulcers. Scientific studies have confirmed that substances extracted from the mushroom can reduce blood pressure, blood cholesterol and blood sugar levels as well as inhibit platelet aggregations [12]. A number of studies revealed that *Pleurotus ostreatus* has numerous diseases curing properties like, anti-cancer activity, immune modulating effects, antiviral, antibiotic and anti-inflammatory activities. *Pleurotus* are excellent producers of lovastatin. *Pleurotus* could be considered as a functional food with natural cholesterol-lowering ability [13]. In oriental medicine *Lentinula edodes* (shiitake) has been used for a wide range of health problems and its curative properties are well attested to in folk medicine [14] (C. The present research is planned to screen for the Thrombolytic activity of three Bangladeshi edible mushrooms, *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Fruiting bodies of three edible mushrooms were collected from National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka-1340, Bangladesh and identified by mushroom development officer, Boucher number #621. The authenticated three edible mushrooms were *Pleurotus ostreatus*, *Ganoderma lucidum*, and *Lentinula edodes*.

2.2 Sample Preparation

The fruiting bodies of the mushrooms were then cleaned and washed to remove any residual compost and dirt by using tap water. The cleaned fruiting bodies were cut and dried at room temperature in the shade and away from direct sunlight for 5 days and then transferred in a hot air oven for 2 days. After drying, the dried samples were grounded into fine power by using

a grinder and kept in a sealed plastic bag for future analysis.

2.3 Sample Crude Extract Preparation

The 80% methanol extraction procedure was used to prepare crude extracts of three authenticated mushrooms. Firstly, 10 grams of powdered mushrooms were suspended with 80% Met-OH in the ratio of 1:15 for *Ganoderma lucidum*, and *Lentinula edodes* and 1:4 for *Pleurotus ostreatus*. The suspended samples were then kept in the dark for 10 days with frequent agitation at room temperature and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron filter paper. The resulting filtrates were then evaporated in the water bath at 60ºC, 60 rpm for 60 hrs until a semi-solid mass of the following colour was obtained; blackish-orange for *Ganoderma lucidum*, and *Lentinula edodes* & yellow for *Pleurotus ostreatus*.

2.4 Sample Preparation for Thrombolytic Activity Study

Two concentrations 400 µg/ml and 800 µg/ml were used. The samples were prepared by scraping off 0.0004 g and 0.0008 g of the solid samples using a spatula which was measured on the analytical balance. The measured samples were then transferred into individual micro centrifuge tube and 1000µl distilled water was added to the tube. 100 µl of this aqueous preparation was added to the micro centrifuge containing the clots to check for thrombolytic activity.

2.5 Study Design

Thrombolytic activity study was performed according to the method described by Prasad et al. [15]. First of all, in commercially available lyophilized streptokinase vial (1 500 000 IU) and 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock solution from which appropriate dilution was made. Five milliliter of venous blood was drawn from the healthy volunteers (n=10) without the history of oral contraceptive or anticoagulant therapy and was distributed (1 mL/tube) to each ten previously weighed sterile micro centrifuge tube and incubated at 37ºC for 90 min to form the clot. After the clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. A volume of 100 µL of the prepared sample was added to each micro centrifuge tube containing pre weighed clot. As a positive control, 100 µL of streptokinase and as a negative control, 100 µL of distilled water were separately added to the control tube numbered. All the tubes were then incubated at 37ºC for 24 hrs, 48 hrs, and 72 hrs and observed clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis.

% of clot lysis =

(wt of released clot /clot wt) × 100

2.6 Statistical Analysis

The mean differences of % thrombolytic activity were analyzed by using student's t test. Two variables were compared with student's t test while three or more variables were analyzed and were compared with one way ANOVA. Null hypothesis testing was done at 5% level of significance. All values are expressed as mean±SD for three replicates.

3. RESULTS

In this *in vitro* thrombolytic analysis, streptokinase and sterile distilled water were used as positive and negative control and compared their clot lysis activities with *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes*. Fig. 1 explores the statistical evaluations of streptokinase (P>0.01) and sterile distilled water (P<0.001) with comparing three incubation periods.

The mean % clot lysis activities of *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes* were affected by doses of sample and by incubation periods. 63.37% clot lysis was found in *Pleurotus ostreatus* where as 34.71% and 29.1 % clot lysis were found in *Ganoderma lucidum* and *Lentinula edodes* respectively at 800 µg/ml dose and at 72 hrs incubation. The mean differences of % clot lysis of *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes* at 400 µg/ml and 800 µg/ml and at 24 hrs, 48 hrs and 72 hrs incubation peroids were statistically significant (P<0.0001). Statistically analyzed relationship between three incubation periods (24 hrs, 48 hrs and 72 hrs) and two doses

(400 µg/ml and 800 µg/ml) of mushrooms are shown in Fig. 2.

The clot lysis activities of *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes* with doses stratified are shown in Fig. 3. Two selected doses form namely 400 µg/ml and 800 µg/ml of edible mushrooms mentioned above were evaluated as thrombolytic agents and were acknowledged their statistical inferences.

The relative clot lysis of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* was also evaluated at 24 hrs, 48 hrs and 72 hrs incubation periods. These dose dependent activities are shown in Fig. 4.

4. DISCUSSION

Atherothrombotic diseases namely myocardial or cerebral infarction occurs due to the development of thrombus that causes hindrance in the passage of vessels and death [4] (Collen D, 1990). Thrombolytic agents that include tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) etc. are used globally for the treatment of these diseases [16]. These drugs are exposed to have several adverse effects associated to atherothrombotic diseases, which lead to further complications [17-19]. At present there is a search for a new thrombolytic agent as the previous ones have some limitations and can lead to fatal consequences.

The results found in this study indicated that *Pleurotus ostreatus* had maximum clot lysis activity i.e. 63.35% at concentration in 72 hrs of incubation. On the other hand *Lentinula edodes* demonstrated minimum clot lysis activity i.e. 29.00% and *Ganoderma lucidum* showed moderate clot lysis activity i.e. 34.16% at 800 µg/ml concentration in 72 hrs of incubation. At 400 µg/ml dose of edible mushrooms, comparatively less clot lysis activity was found with different incubation periods. In our findings it was clearly observed that the mushroom extracts enhanced clot lysis in dose dependent manner along with the incubation time factor.

According to Maafi Rizwana Islam et al. 2015 [20], eleven selected phytochemicals were found in the extracts of the fruiting bodies of *Ganoderma lucidum* with polyphenols, flavonoids, tannins, coumarins, vit-C, and anthocyanins found in highest concentration category. Except polyphenols, *Lentinula edodes* showed the rest of 10 selected phytochemicals with vit-C and tannin found in maximum concentration category. Saponins, coumarins, and anthocyanins were absent in the fruiting bodies of *Pleurotus ostreatus* while steroids, terpenoids, cardiac glycosides were found in uppermost concentration category. Studies revealed that the thrombolytic activity was probably due to the phytoconstituents present in the mushroom samples.

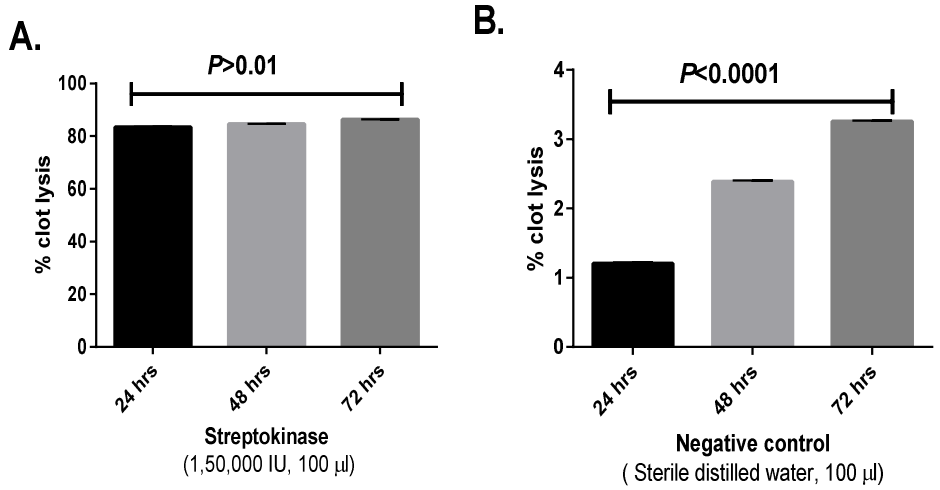

Fig. 1. Effects of incubation periods on clot lysis activity of streptokinase and sterile distilled water. (A) No statistically significant mean difference of % clot lysis by positive control (P>0.01) and (B) mean differences of % clot lysis amidst 24 hrs, 48 hrs and 72 hrs incubation of negative control were statistically significant (P<0.0001)

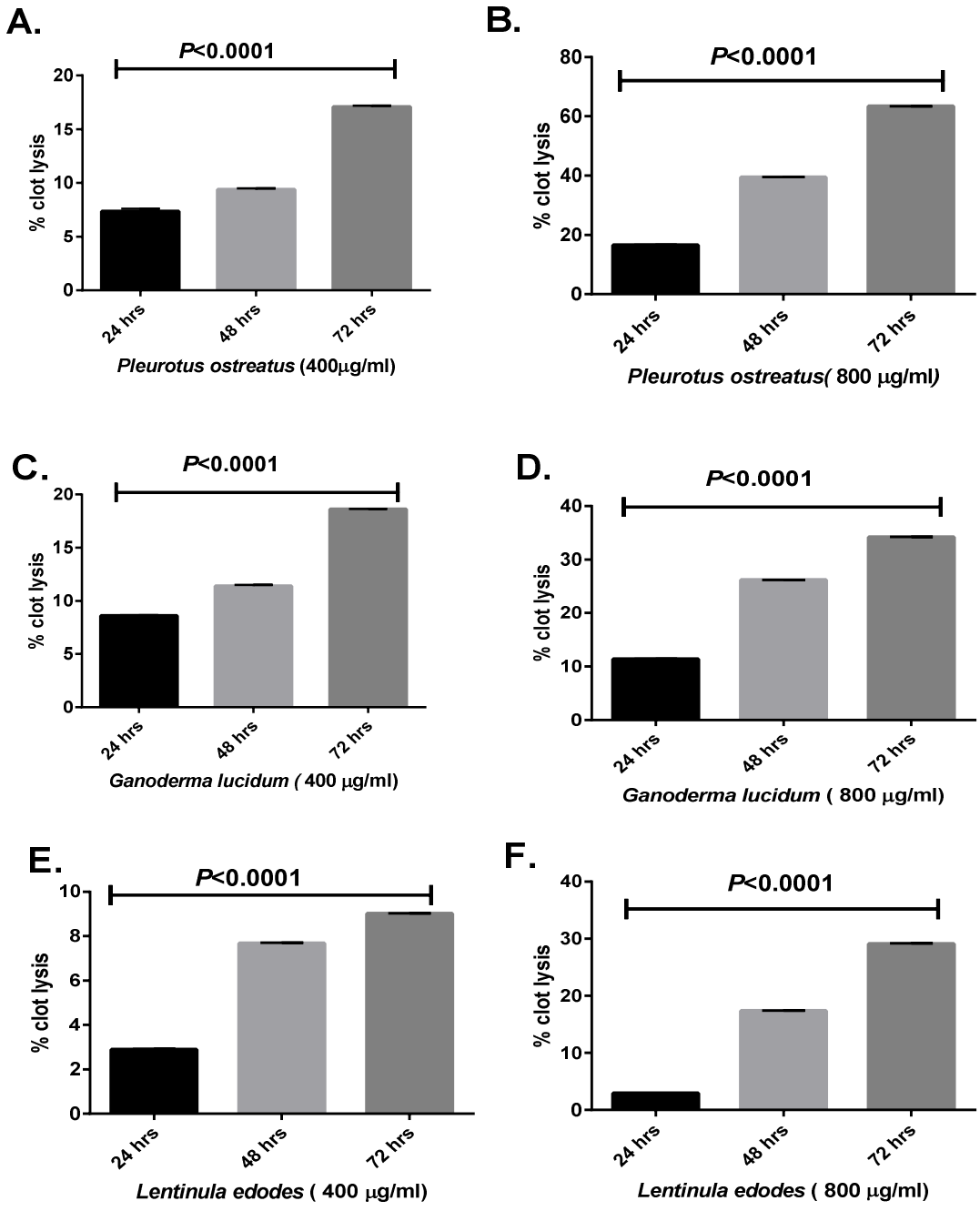

Fig. 2. Effects of incubation periods on clot lysis activities of *Pleurotus ostreatus, Ganoderma lucidum* and *Lentinula edodes*. (A) and (B) explores the clot lysis of 400 µg/ml and 800 µg/ml of *Pleurotus ostreatus*. The mean differences of % clot lysis of 400 µg/ml (P<0.0001) and 800 µg/ml (P<0.0001) were statistically significant. In (C) and (D), mean clot lysis of *Ganoderma lucidum* with two doses of 400 µg/ml (P<0.0001) and 800 µg/ml (P<0.0001) were statistically underlined. (E) and (F) shows the mean clot lysis of *Lentinula edodes* with two doses of 400 µg/ml (P<0.0001) and 800 µg/ml (P<0.0001)

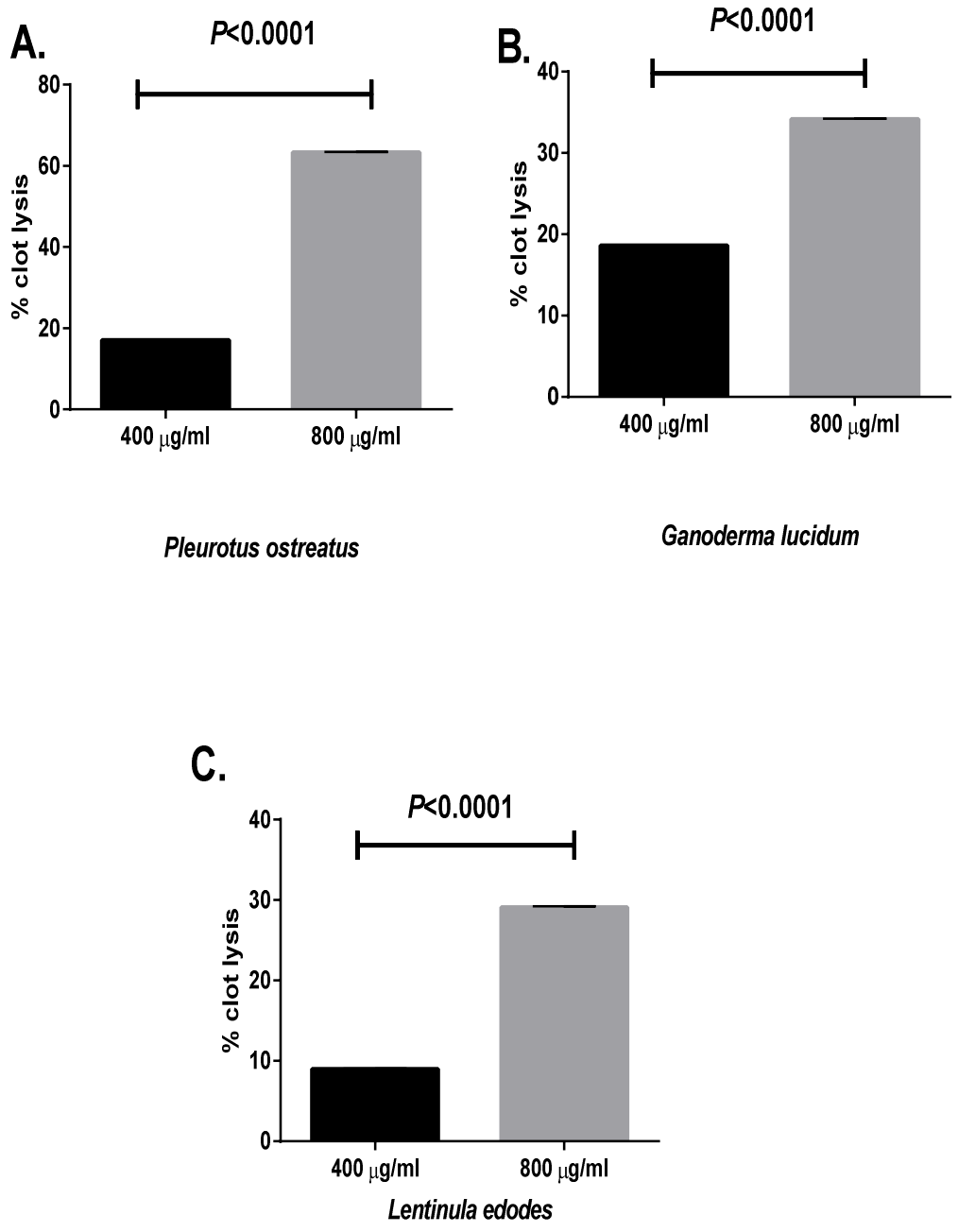

Fig. 3. Dose dependent clot lysis activity of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* extracts. (A) The mean difference of % clot lysis between 400 µg/ml and 800 µg/ml doses of *Pleurotus ostreatus* was significant (P<0.0001). (B) Statistically significant inference (P<0.0001) was documented in case of the mean difference of % thrombolytic activity by *Ganoderma lucidum*. (C) *Lentinula edodes* showed significant % clot lysis activity (P<0.0001)

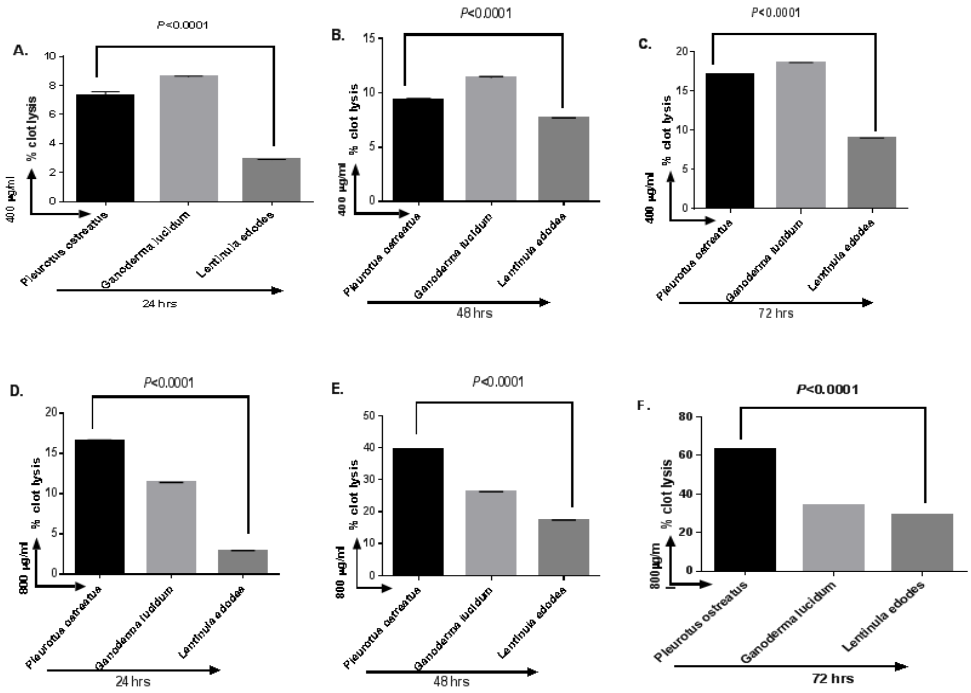

Fig. 4. Comparative clot lysis activities of three selected edible mushrooms with their three incubation periods (24 hrs, 48 hrs and 72 hrs) and two doses (400 µg/ml and 800 µg/ml) stratifications.(A), (B), (C) explore the clot lysis of 400 µg/ml of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* at 24 hrs, 48 hrs and 72 hrs respectively. The mean differences of clot lysis of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* were statistically very significant (*P*<0.0001). (D), (E) and (F) elucidate the clot lysis of 800 µg/ml of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* at 24 hrs, 48 hrs and 72 hrs one-to-one. Statistically significant (*P*<0.0001) mean differences of clot lysis of *Pleurotus ostreatus*, *Ganoderma lucidum* and *Lentinula edodes* were found

5. CONCLUSION

Based on the results obtained from the study, thrombolytic activities of three edible mushrooms are doses and incubation dependent. It can also be concluded that the methanolic extracts of the three mushrooms can be successfully applied in the development of more potent and efficient thrombolytic agents as they showed reasonable thrombolytic activity. However, further investigation is required to confirm their pharmacological activity and thereby utilizing them as useful medicinal plant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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