

## **Haemostatic effect of aqueous extract of mushroom (*Ganoderma lucidum*)**

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### **ABSTRACT**

The study investigated the haemostatic effect of aqueous extract of Mushroom (*Ganoderma lucidum*) in rats using tail bleeding time and blood clotting time. Rats were divided into four groups of five rats (n=5) animals. Group I served as normal control, Group II received 200mg/kg b w, group III were given 400mg/kg b w while Group IV were administered with 800mg/kg b w of the extract intra-peritoneally for a period of ten days respectively. The findings of this study revealed a significantly decreased ( $P<0.05$ ) bleeding time and clotting time at all doses 200, 400 and 800mg/kg b w after 3<sup>rd</sup> day, with a non-significantly decreased ( $p>0.05$ ) time observed after 6<sup>th</sup> and 10<sup>th</sup> day of administration plant extract respectively. In conclusion, this justifies the traditional use of *Ganoderma Lucidum* in stoppage of blood bleeding.

**Key words:** Bleeding time, clotting time, *Ganoderma lucidum*, haemostasis, rats,

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### **INTRODUCTION**

Haemostasis is a process that prevents excessive blood loss in the body. The haemostatic system represents a delicate balance between pro-coagulant, anticoagulant mechanisms allied to a process of fibrinolysis [1]. There are five major components involved in the haemostatic system, viz: platelets, coagulation factors, coagulation inhibitors, fibrinolysis and blood vessels [2]. Haemostasis is a life saving process and therefore exploration of compounds that facilitate the process is of medicinal importance [3]. Plants are important sources of many biologically active compounds. Plants used in traditional medicine provide an interesting and still largely unexplored source for the development of new drugs [4]. *Ganoderma lucidum* (Fr.) Karst, a popular medicinal mushroom, has been used in traditional medicines in many Asian countries. It has been widely used as medicine to promote health and longevity in China for thousands of years [5]. Studies have shown that *Ganoderma lucidum* have different active constituents. Some of the chemical constituents of *G. lucidum* include polysaccharides, proteins, nucleosides, fatty acids, sterols and triterpenes [6]. In East Asia, the fruiting body of the fungus *Ganoderma lucidum* has been used for centuries. It has long been used as a folk medicine to treat various human diseases such as cancer, hypertension, hepatitis, nephritis and so on [5]. The aim of this study is to investigate the haemostatic activity of aqueous extract of *Ganoderma lucidum* in Wistar rats.

## MATERIALS AND METHODS

Materials used include: filter papers, electronic stopwatch, disposable lancet, non-heparinized capillary tubes, de-ionized water, beaker, measuring cylinder and Gallen mettle balance (P162, Switzerland), Water Bath or Heat Block standardized to 37<sup>0</sup> C and Thermometer.

### Plant collection and extraction

The fruiting body of *Ganoderma lucidum* was collected from around Ahmadu Bello University, Zaria promises and environs in the Month of November, 2009. The plant was taken to the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University, and Zaria where a voucher number was deposited. The plant was dried using a drying oven at 50 <sup>0</sup>C. It was then pounded into a coarse powder using a laboratory mortar and pestle. About 500 g of the powder was obtained and cold macerated with 2 liters of distilled water for 48 hours. The product was filtered using Watmann sized filter paper 1 to obtain a dark brown mass that was stored in air tight container until it was reconstituted.

### Animal management and care

Strains of albino wistar rats of both sexes weighing between 150-234 g were procured from the Animal House of the Department of Human Physiology, Ahmadu Bello University, and Zaria. The animals were maintained under laboratory condition in order to acclimatize. They were allowed to have free access to water *ad libitum* prior to the commencement of the work.

### Experimental protocol

A total of sixteen (16) apparently healthy strains of albino rats were used. They were randomly assigned into four groups of four (4) rats each as follows:

Group I served as the control and received 1ml of distilled water

Group II were treated with 200 mg/kg b w of *G. lucidum*

Group III were treated with 400 mg/kg b w of *G. lucidum*

Group IV received 800 mg/kg b w of *G. lucidum* respectively.

All administration was done orally by gavage for a period of ten (10) days.

### Determination of bleeding time

This was determined using a modified Duke method [7]. Briefly, the tail of each animal from the respective group was disinfected by cleaning with methylated spirit. The tail of the animal was held from the perforated spaces in each cage, and the tip was quickly cut using a disposable lancet and the stopwatch was started as soon as bleeding started. The cut was dabbed with filter paper every 15 s until the paper no longer stained red with blood. Bleeding time was then taken as the time when the blood stopped flowing from the cut. The bleeding time was determined by counting the number of spots the blood blotted off on the filter paper. The spot was then added up; it was multiplied by the interval by which the blood was blotted off that is 30 seconds. This is then multiplied by 60 to convert it to minutes.

### Determination of clotting time

This was carried by method described by Cole, (1987) [8]. Briefly, the tail of the animal from each group was cleaned and disinfected with methylated spirit and then cut with a disposable lancet. The tip of the tail of each animal was immediately directed into four glass test tubes that had previously been warmed and maintained at 37°C and the tubes immediately placed in a 37°C water bath to mimic the temperature of the internal environment. The stopwatch was started immediately the blood was delivered into the glass test tubes to determine the bleeding time and after 30 seconds the end of the test tubes were cut a pair of scissors and note the time when the blood appear gelatinous. The clotting time was taken as the average of the times blood clotted in the four tubes.

### Statistical Analysis

The results of bleeding and clotting time were expressed as mean  $\pm$  SEM. The data were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was done using SPSS version 17.0 software. The values of  $p < 0.05$  were considered as significant [9].

## RESULTS AND DISCUSSION

## Results

**Effects of aqueous extract of *Ganoderma lucidum* on bleeding time in rats**

There was a significantly decreased ( $p < 0.05$ ) bleeding time in the animals treated with all doses 200, 400 and 800 mg/kg b w of *G. lucidum* after day 3 when compared to the control group (Table 1). While administration of 200, 400 and 800 mg/kg b w showed no significant decrease ( $p > 0.05$ ) on bleeding time after the 6<sup>th</sup> and 9<sup>th</sup> day respectively when compared to the control group (Table 1).

**Effects of aqueous extract of *Ganoderma lucidum* on clotting time in rats**

There was a significant decrease ( $p < 0.05$ ) on clotting time in the group treated with 200 and 800 mg/kg b w of the extract after the 3<sup>rd</sup> day when compared to the control group (Table 2). While administration of 400 mg/kg b w of the extract showed non-significant decrease ( $p > 0.05$ ) on the clotting time in the animals after the 3<sup>rd</sup> day when compared to the control group (Table 2). However after the 6<sup>th</sup> day, administration of the extract at a dose of 200 mg/kg b w significantly reduced ( $P < 0.05$ ) the clotting time, with a non-significant change ( $p > 0.05$ ) observed in the groups treated with 400 and 800 mg/kg b w of the extract when compared to the control group (Table 2).

**Table 1: Effects of aqueous extract of *Ganoderma lucidum* on mean ( $\pm$  SEM) bleeding time in rats**

Treatment given	Mean ( $\pm$ SEM) (Minutes) Day 3	Mean ( $\pm$ SEM) Minutes) Day 6	Mean ( $\pm$ SEM) Minutes) Day 9
Control + distilled water	6.40 $\pm$ 0.19 <sup>a</sup>	3.50 $\pm$ 0.35	3.10 $\pm$ 0.60
200 mg/kg b w	2.70 $\pm$ 0.122 <sup>a</sup>	2.70 $\pm$ 0.122 <sup>ns</sup>	3.0 $\pm$ 0.42 <sup>ns</sup>
400 mg/kg b w	3.40 $\pm$ 0.19 <sup>a</sup>	4.60 $\pm$ 0.78 <sup>ns</sup>	4.60 $\pm$ 0.73 <sup>ns</sup>
800 mg/kg b w	2.80 $\pm$ 0.339 <sup>a</sup>	3.50 $\pm$ 0.22 <sup>ns</sup>	1.90 $\pm$ 0.187 <sup>ns</sup>

<sup>a</sup>  $p < 0.05$  is statistically significant when compared to control group, while <sup>ns</sup> = not significant when compared to the control group

**Table 2: Effects of aqueous extract of *Ganoderma lucidum* on mean ( $\pm$  SEM) clotting time in rats**

Treatment given	Mean ( $\pm$ SEM) (Minutes) Day 3	Mean ( $\pm$ SEM) (Minutes) Day 6	Mean ( $\pm$ SEM) (Minutes) Day 9
Control + distilled water	8.30 $\pm$ 0.20	6.90 $\pm$ 0.58	4.50 $\pm$ 0.52
200 mg/kg b w	5.50 $\pm$ 0.16 <sup>a</sup>	3.90 $\pm$ 0.10 <sup>a</sup>	3.60 $\pm$ 0.40 <sup>ns</sup>
400 mg/kg b w	6.80 $\pm$ 0.26 <sup>ns</sup>	6.20 $\pm$ 0.25 <sup>ns</sup>	5.70 $\pm$ 0.72 <sup>ns</sup>
800 mg/kg b w	5.60 $\pm$ 0.37 <sup>a</sup>	8.50 $\pm$ 0.57 <sup>ns</sup>	2.80 $\pm$ 0.41 <sup>ns</sup>

<sup>a</sup>  $p < 0.05$  is statistically significant when compared to control group, while <sup>ns</sup> = not significant when compared to the control group

## DISCUSSION

Haemostasis which is the arrest of blood loss from severed blood vessels and the maintenance of blood fluidity involves coagulation and fibrinolysis. A wound or cut on blood vessels causes vasoconstriction and thrombin activation which are then accompanied by adhesion and platelet activation, fibrin formation from circulating fibrinogen and coagulation inactivation mechanism [10-11]. The present study was carried out to determine the potentials of *G. lucidum* on the haemostatic mechanism, with primary interest on how it affects bleeding and clotting time respectively. Bleeding time evaluates the vascular and platelet responses with haemostasis [12] [3], whereas the clotting time measures the intrinsic clotting factors (I, II, V, VIII, IX, X, XI and XII). Clotting time test is a qualitative measurement of factors involved in the intrinsic pathway [7]. Therefore, deficiency in the factors of the intrinsic pathway (I, II, V, VIII, IX, X, XI, and XII) will affect the result. From the results obtained in this work, the extract showed a significantly decreased ( $p < 0.05$ ) clotting time, reflecting that there was an increase in one or more of the clotting factors involved in the intrinsic pathway. Plasma fibrinogen which was not measured in this study has been known to facilitate the rate of fibrin polymer formation which ultimately leads to more effective clot formation [2] as was observed in present work. The results recorded in this study are in consonance with the reports of Okoli *et al.*, (2007) [13] on the haemostatic activities of the leaf extract of *Aspilia Africana* which arrested bleeding from fresh wounds by reducing both bleeding and clotting times. Bamidele *et al.*, (2010) [14] have also reported that methanol leaf extract of *Ageratum conyzoides* significantly reduced bleeding and clotting times in albino wistar rats. The preliminary phytochemical screening of *Ganoderma lucidum* carried out by Mohammed *et al.*, (2009) [15] showed the presence of flavonoids, alkaloids and tannins, many of which are biologically active phytochemicals. Tannins have been implicated in the haemostatic activity of plants where they arrest bleeding from damaged or

injured vessels by precipitating proteins to form vascular plugs (Okoli *et al.*, 2007; Bamidele *et al.*, 2010). Therefore, the haemostatic mechanism of *G. lucidum* may be related to the presence of these phytochemicals.

### CONCLUSION

In conclusion, the aqueous extract of *Ganoderma lucidum* possessed haemostatic property as evidenced by its significant reduction on bleeding and clotting time in rats.

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