

ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF THE ETHANOLIC EXTRACT OF THE MUSHROOM *GANODERMA APPLANATUM*

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ABSTRACT

Objective: The ethanolic extract of the mushroom, *Ganoderma applanatum* was investigated for the presence of secondary metabolites, anti-inflammatory and analgesic activities.

Material and Methods: The extract was subjected to phytochemical screening for the identification of secondary metabolites. Using intraperitoneal doses of 150, 300, 600 and 1200 mg/kg body weight, in vivo anti-inflammatory screening was carried out in rats using the rat paw oedema test while analgesic activity of the extract was done in mice using the hot plate test.

Results: The study shows that the extract has an LD₅₀ of 3273 mg/kg and the presence of anthraquinones, flavonoids and steroids. Saponins and tannins were absent. Dose and time dependent suppression of egg-white induced oedema in the rat paw test was observed, with maximum percentage suppression occurring at 600mg/kg (59.2%). The extract also significantly prolonged the reaction time in mice to noxious thermal stimuli.

Conclusion: *Ganoderma applanatum* has potential as a source of useful analgesic and anti-inflammatory principles.

Keywords: *Ganoderma applanatum*, anti-inflammatory, analgesic, mushroom

1. INTRODUCTION

Mushrooms are invaluable sources of useful therapeutic agents [1] in addition to their increasing use as functional foods for the prevention of diseases such as diabetes mellitus, hypertension and cancer [2,3]. This is due to the presence of useful nutrients and secondary metabolites [4]. Antineoplastic polysaccharides sourced from mushrooms such as lentinan and schizophyllan are currently approved and being marketed for the management of various cancers [5]. Mushrooms contribute to the largely untapped bio-diversity resource available to indigenous populations for the development of useful therapeutic agents beneficial for improving health [6], and there is immediate need to research Nigerian mushrooms for potential sources of useful pharmacological agents.

Ganoderma applanatum is a basidiomycete commonly found growing on dead wood in both temperate and tropical regions of the world [7]. It is a perennial macrofungi with a very hard fruiting body that is devoid of stalk. It adds a new layer of pores every year giving a typical light green rings on the fruiting body. It is not considered an edible mushroom by inhabitants of Jos, Nigeria.

Members of the genera *Ganoderma* have a wide range of pharmacological activities. Useful antineoplastic, antioxidant and hypoglycaemic principles have been identified and isolated [8,9]. Hepato-protective principles such as the optically active ganoderic and ganosporeric acids have been isolated from *G. lucidum* [10].

Inflammation is increasingly being implicated in the pathophysiology of many chronic conditions including diabetes mellitus [11], cancers, cardiovascular and musculoskeletal disorders [12]. Currently available anti-inflammatory drugs have limitations bordering on costs and toxicities such as gastro-intestinal ulcerations [12].

This work was therefore carried out to investigate locally growing *Ganoderma applanatum* for potential anti-inflammatory and antinociceptive activities.

2. MATERIALS AND METHODS

2.1 Collection and preparation of mushroom extract

Whole mushrooms of *Ganoderma applanatum* found growing in the wild on stumps of eucalyptus trees were collected between November 2010 and June 2011 in Jos-Nigeria. They were identified with the aid of web-based pictures and reference materials [13]. They were dried to constant weight at 35°C in a hot air oven after which they were crushed to a coarse powder using a mortar and pestle, 100g of which was cold-extracted by maceration with 300ml of absolute ethanol in a 500ml conical flask, with constant shaking, at room temperature for 72 hours. At the end of the extractive process, the mixture was filtered under suction and the resulting filtrate evaporated over a water bath at 45°C yielding a dark brown gelatinous crude extract which was stored at 4°C until required for use.

2.2 Experimental animals

Sprague-Dawley rats and albino mice of the Wister strain of mixed sex were procured from the Animal House facility of the University of Jos for the experiment. They were kept in standard cages with access to standardized feed and water *ad libitum* under 12 hour light-dark cycle. Clearance for the use of experimental animals was sought and obtained from the Animal Ethics Committee of the Department of Pharmacology, University of Jos.

2.3 Phytochemical screening of extract

The ethanolic extract of the whole mushroom *Ganoderma applanatum* was investigated for the presence of alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroids and tannins, according to the protocols described by [14].

2.4 Determination of Mean Lethal Dose of the extract

The LD₅₀ in mice was determined according to the method described by Lorke [15]. Briefly, 9 mice were divided into 3 groups of 3 mice each, representing three geometrically increasing dose points of 10, 100 and 1000 mg/kg of the extract, administered intraperitoneally. Animals were observed for mortality within each group over a 24 hour period. The LD₅₀ was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

2.5 Test of anti inflammatory activity

The method described by [16] was used, with minor modifications. Briefly, 25 rats (120-150g) were divided into 5 groups of five animals. One group served as a negative control (normal saline) and another as positive control (Indomethacine 3 mg/kg IP). The 3 remaining groups received separately 300, 600 and 1200 mg/kg of the extract intraperitoneally. Acute inflammation was induced by sub-plantal injection of 0.1ml of egg albumin into the right hind paw. Extracts were administered 30 minutes before induction of oedema. Paw circumference was measured using a vernier caliper 30 minutes before, immediately after and at 0.5, 1, 2, 4 and 8 hours after induction. Experimental groups were compared with the vehicle and reference drug control groups.

2.6 Analgesic screening

Twenty five mice were divided into 5 groups of five mice each, representing 3 dose points (150, 300 and 600 mg/kg) of extract and 2 control groups (reference drug and inert vehicle). Screening of analgesic activity was done using the thermal method [17]. Time taken for each mouse in each group to react (jumping, licking of paws) to the noxious stimuli was measured in seconds. Extract-treated groups were compared with negative and positive control groups. Mean group values were computed.

2.7 Statistical Analysis

Mean values were computed and expressed as standard error of mean. Test of significance was done using the Students' T-test, with test groups being compared with negative control groups.

3. RESULTS

The LD₅₀ in mice was found to be 3273 mg/kg via the intraperitoneal route.

Phytochemical screening shows that the extract had relatively high content of alkaloid, anthraquinones, flavonoids, and steroids (Table 1). However, saponins and tannins were absent.

Table 1: Phytochemical Constituents of the Ethanolic Extract of the Whole Mushroom *G. applanatum*

Bioactive principles	Results
Alkaloids	+++
Saponins	-
Flavonoids	+++
Tannins	-
Carbohydrates	++
Anthraquinones	+++
Steroids	+++

Key: - = Absent + = Present

The highest suppression of oedema was observed at a dose of 600 mg/kg body weight (Table 2).

TABLE 2: Effects of Ethanollic Extract of *Ganoderma applanatum* on Egg-White Induced Rat Paw Size

Treatment	Dose (mg/kg)	Paw size (mm)					
		30 min	60 min	120 min	180 min	240 min	300 min
Normal saline		0.51±0.01	1.10±0.03	1.15±0.01	1.19 ± 0.03	1.24 ± 0.02	1.25±0.02
Indomethacin	5.0	0.50±0.01	0.91±0.02 ^b	0.82±0.01 ^b	0.73±0.02 ^b	0.70±0.01 ^b	0.61±0.01 ^b
EGA	300.0	0.51±0.01	0.92±0.02 ^b	0.72±0.08 ^b	0.62±0.04 ^b	0.58±0.03 ^b	0.51±0.02 ^b
EGA	600.0	0.50±0.01	0.84±0.05 ^b	0.66±0.05 ^b	0.61±0.05 ^b	0.61±0.04 ^b	0.51±0.02 ^b
EGA	1200.0	0.50±0.01 ^a	0.82±0.08 ^a	0.73±0.01 ^b	0.58±0.04 ^b	0.56±0.05 ^b	0.57±0.04 ^b

Data are mean ± SEM; ^ap<0.05, ^bp< 0.01; n = 5 animals per group EGA = Extract of *G.applanatum*

The extract produced a significant and dose-dependent elongation in reaction time (analgesic activity) in the hot plate analgesic screening model (Table 3).

TABLE 3: Effects of the Ethanollic Extract of *Ganoderma applanatum* on Reaction Time Following Thermal Nociceptive Stimuli

Treatment	Dose	Reaction time (seconds)					
		30min	60min	90min	120min	150min	180min
Normal saline	0.1ml	8.33±1.20	10.33±0.33	10.00±0.58	8.33±0.88	9.33±0.67	10.00±0.58
Pentazocine	1.5mg/kg	34.33±2.03 ^b	38.00±1.15 ^b	42.33±1.76 ^b	54.33±3.48 ^b	64.00±2.65 ^b	69.00±3.46 ^b
EGA	150mg/kg	16.67±0.88 ^b	22.00±0.58 ^b	25.67±1.20 ^b	29.00±0.58 ^b	40.33±2.60 ^b	47.67±1.86 ^b
EGA	300mg/kg	20.33±0.88 ^b	27.33±0.67 ^b	35.67±2.40 ^b	40.33±2.60 ^b	44.33±3.18 ^b	53.33±4.41 ^b
EGA	600mg/kg	28.00±1.15 ^b	34.67±1.20 ^b	49.67±3.28 ^b	68.67±2.40 ^b	80.33±2.60 ^b	85.33±4.91 ^b

Data are mean ±SEM ^bP< 0.01; n = 3 animals per group EGA = Extract of *G.applanatum*

4. DISCUSSION

The LD₅₀ of the ethanollic extract of the whole mushroom *G. applanatum* in mice via the intraperitoneal route was found to be 3873 mg/kg according to the Locke method [15]. This makes the extract slightly toxic based on the Gosselin, Smith and Hodge scale [18]. Mushrooms are known to contain highly toxic principles capable of harming man, thus the classification of mushrooms into edible and inedible types [19, 20].

Phytochemical evaluation of the extract showed the presence of secondary metabolites such as steroids, anthraquinones, flavonoids and alkaloids (Table 1). Principles such as flavonoids and steroids are known to have antioxidant and anti-inflammatory properties. This agrees with the findings of Just *et al.* [21] that flavonoids, saponins and steroids possess analgesic and anti-inflammatory properties. The presence of secondary metabolites such as alkaloids, steroids and anthraquinones suggests the potential of locally growing mushrooms becoming important sources of new drug templates.

The extract exhibited significant dose and time-dependent anti-inflammatory activity in the acute model of inflammation involving the induction of oedema in rat hind paw, comparable to the reference drug indomethacin (Table 2). Maximum suppression of oedema (59.2%) occurred at a dose of 600 mg/kg. The anti-inflammatory effect induced was sustained for a duration reaching 5 hours. Irritant-induced inflammation occurs in two qualitatively distinguishable phases [22]. The early phase begins within minutes of phlogistic challenge due to the release of biogenic amines such as histamine, while the latter phase involves the synthesis of prostaglandins. Drugs with known cyclooxygenase inhibitory activity such as Non-Steroidal Anti-inflammatory Drugs (NSAID) suppress this later phase of oedema formation [22]. Egg white is an alternative phlogistic agent that triggers the release of inflammatory process via release of mediators [23].

The extract potently and significantly prolonged reaction time in mice subjected to thermal stimuli, indicative of an analgesic effect, comparable with the opioid agonist pentazocine. The hot plate test of nociception screens for substances with central nervous system activity [24]. The hot plate test however, does not discriminate between central analgesics and muscle relaxants/sedatives, which also prolong reaction time in the hot plate test (Vogel, 2008).

5. CONCLUSION

The present study establishes the analgesic and anti-inflammatory activities of *G. applanatum* harvested from Jos-Nigeria. Further characterization of these actions including the active principles involved and their mechanisms of action are the subject of ongoing research.

6. ACKNOWLEDGEMENTS

Mr. Vambe Moses Tavershima is appreciated for help with aspects of the laboratory work.

7. REFERENCES

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