Effects of *Ganoderma lucidum* on Chemical-Induced and Bacterial-Infected Corneal Ulceration of Rabbits' Eyes

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Abstract

Background

Corneal ulcerations are risks to blindness, and their treatment presents huge challenges, in part due to increased resistance to anti-bacterial drugs, accessibility and costs issues, particularly in developing countries. In this study, we investigated the pharmacological effect of *Ganoderma Lucidum (G. lucidum)*, on chemical-induced and bacterial-infected corneal ulceration of rabbits' eyes.

Methods

This was a randomized-controlled experiment of 16 healthy New Zealand rabbits, which were randomly assigned into four groups (A – D). The right eye corneas were injured using a drop of 1 molar sodium hydroxide and followed by infection with *Pseudomonas aeruginosa* after 12 hours, in all groups except group A. Treatment with extracts of *G. lucidum* for groups A and B, the standard treatment protocol for group C, and atropine alone for group D commenced after 24 hours and continued every four hours for seven days. All data collected, pre- and post- tests, were analyzed at $\alpha = 0.05$ using Wilcoxon signed-rank test for correlated variables and Mann-Whitney U test for independent variables.

Results

G. lucidum has significant clinical effects (47%) on corneal injury and ulcers (92%). The healing effects observed at the 168th hour were significantly better than those observed at the 24th hour ($p < 0.05$). Although the differences between the healing effects of the standard treatment and the extracts were not significant ($p > 0.05$), the mean effects size was clinically significant (31%).

Conclusion

This study demonstrates the potential antimicrobial potential of *G. lucidum*. *G. lucidum* may provide an alternative treatment option for chemical-induced and bacterial-infected corneal ulcers, particularly in resource-constrained countries.

Background

Corneal ulcers are estimated to cause 7.2% of corneal blindness [1], and are thus a significant contributor to blindness. In Kenya, corneal injuries have been reported to account for an estimated 2.7% of corneal blindness [2]. While corneal injuries are common, access to treatment and medical care is limited, and this may be due to a lack of awareness of treatment options, accessibility or cost [3]. Lack of appropriate and prompt management of ulcers leads to poor wound healing and subsequent formation of scars, resulting in partial or total vision loss.

Antibiotics act by interfering with the metabolic processes or with the organism's structures. However, there are growing concerns about the number of drug resistant bacterial strains [4]. Thus, many disease conditions are

becoming more difficult to treat due to the emergence of antibiotic-resistant bacteria [5]. In 2010, the World Health Organization (WHO) recommended that all countries implement control procedures to mitigate the effects of multi-drug resistant bacteria [6]. It emphasized the urgent need for alternative therapies to be identified against drug-resistant microorganisms in low- and middle-income countries, such as Kenya. In addition, Kenya has been reported to have a high utilization rate of unorthodox medical treatment due to challenges with access to orthodox medicine, cost and cultural beliefs [3].

A mushroom is a fruiting body of fungi that is non-photosynthetic and feeds on organic matter and plant organisms [7]. *Ganoderma lucidum (G. lucidum)* is a type of mushroom which has basidiocarp, mycelia and spores that contain approximately 400 different bioactive compounds [8]. *G. lucidum* as a mushroom is in the family of Ganodermataceae, a family consisting of a large group of tree fungi of the genus Ganoderma. *G. lucidum* has been classified into Kingdom Fungi, Phylum Basidiomycota, Class Basidiomycetes, Sub-class Homobasidiomycetes, Order Polyporales, Family Ganodermataceae, Genus *Ganoderma* and Species *lucidum* [9, 10]. The mushroom has been reported to have several pharmacological effects, such as immunomodulation, analgesic, anti-atherosclerotic, anti-inflammatory and antibacterial, among other medicinal benefits to the human body [8]. The antibacterial effect of *G. lucidum* has been shown to have a healing effect on non-ocular related inflammatory injuries and infective ulcers in other parts of the human body [11]. Many ulcerative conditions are becoming difficult to manage due to the increasing numbers of drug-resistant bacteria strains such as *Staphylococcus aureus, Pseudomonas pyocyanea, Streptococcus pneumoniae, Pseudomonas aureginosa* [5, 12]. *G. lucidum* has been proven to be effective in managing bacterial infections that have shown resistance [13], with reports indicating it to be an effective alternative to treating bacterial infections and inflammatory injuries to the human body [14]. *G. lucidum* is commonly available in Kenya [7]. This study aimed to investigate the effects of *G. lucidum* on chemical-induced and bacterial-infected corneal ulcers in rabbits' eyes. Furthermore, the study aimed to compare the effects of *G. lucidum* with those of standard orthodox treatment approaches to chemical and bacterial corneal ulcers to establish a possible relatively cheaper alternative and/or complementary treatment to these corneal insults.

Methods

Study design

This randomized-controlled experiment was conducted in the animal house of Masinde Muliro University of Science and Technology (MMUST), Kakamega, Kenya, which is located in the western part of Kenya, 1 km from Kakamega central town. In this experiment, 16 healthy, New Zealand rabbits were pre-tested for normal physiological functions, such as the eyelid, lacrimation and corneal statuses, and also re-tested 12 hours after the introduction of corneal injury for changes from the baseline physiological findings. The rabbits were labelled 1 to 16 and randomly assigned to four groups as follows: A – Experiment 1; B – Experiment 2; C – Positive control and D – Negative control, this randomization and assignment to groups was without bias to gender or weight of the rabbits.

Group A: Experimental group 1. The rabbits were treated with *G. lucidum* extracts following exposure of their cornea to a chemical-induced injury.

Group B: Experimental group 2. The rabbits were treated with *G. lucidum* extracts following exposure of their cornea to a chemical injury and the subsequent infection with P*seudomonas aeruginosa.*

Group C: Positive control group. The rabbits were treated with a standard treatment approach for corneal ulceration, that being two hourly drops of fluoroquinolones (Ciprofloxacin), eight hourly atropine sulphate and four hourly betamethasone ophthalmic formulations.

Group D: Negative control group. The rabbits received eight hourly drops of atropine sulphate, both as a placebo and to relieve the pain, without the intention of providing normal treatment.

Data collection instruments

Instruments for data collection included the Pen torch, a magnifying loupe, Keeler® ophthalmoscope, 2 ml hypodermic syringes and needles, sample tubes, micro pipette and pipette. Others included beakers, spatulas, weighing scale, water bath, mortar and pestle, a sterilized laboratory grinder, Turtle stand, What-man® filter paper, Dettol®, gloves, a small bucket with a cover, as well as Methylated spirit, Vaseline® jelly, a razor blade and cotton wool. Dean stark apparatus, clean distilled water, double pocket condenser, a 5L-3-necked round bottomed flask, Ganoderma mushrooms and a camera were also required. Drugs used for this experiment were; Crude extract of *G. lucidum*, Tetracaine Hydrochloride 0.5%, Atropine sulphate 1%, Probeta-N® (Betamethasone-Neomycin), Ceprolen® (Ciprofloxacin), Nepuscein® (fluorescein) strips, Bovitam®, Amilyte®, Piperazine®, Ketamine hydrochloride and 1 molar Sodium hydroxide.

Study procedure

Systemic anesthesia (1 ml/50mg of ketamine hydrochloride) was injected intramuscularly into the 16 rabbits in groups **A, B, C and D**, and five minutes later, two 2 drops (1 ml of 0.5%) of Tetracaine hydrochloride (topical anesthesia) was instilled in their right eye only. After another 3–5 minutes, a corneal sensitivity test was performed using cotton swabs test to ensure that the right eye cornea of each rabbit was fully anaesthetized. For confirmation, the same procedure was done on the left eye to verify their response to the cotton tips rubbed on the cornea. Following confirmation of corneal anesthesia in the right eye, 1 drop of 0.2ml/1molar sodium hydroxide (NaOH) was gently instilled on the central corneal surface using a cotton swab, while the corneal peripheral and conjunctiva areas were guided from the spread of the chemical with another set of cotton wool swabs placed around the limbus of the right eye, while the chemical was being allowed to soak into the corneal epithelial cells.

This procedure was performed under minimal magnification, using a 10x magnifying loupe. The affected central cornea area was then assessed for successful induction of injury by applying a stain with a swipe of fluorescein strip and observing with the blue illumination of a Keeler ophthalmoscope under 20x magnification. The right eyes were padded and left for 12 hours and then observed. Ocular manifestation (signs of inflammations and possible infections) following the induction of chemical injury was observed after 24 hours. The following parameters were used to assess for ocular manifestations: eyelid and conjunctival status, lacrimation, epithelial disruption, corneal fluorescein staining, photophobia (using a flash light), corneal edema and infiltrates. The ocular manifestations were consistently graded on a scale of 0 to 5 (Normal/no change observed = 0, Very mild = 1, Mild = 2, Moderate = 3, Severe = 4 and Very severe = 5) [15]. The rabbits in groups B, C and D were then infected with two drops of a laboratory prepared solution of *P. aeruginosa* and padded for

another 24 hours, after which they were examined for signs of active inflammation and infection, with heavy mucopurulent discharges and stuck eyelids being evident.

After 24 hours, treatment commenced with the animals in groups A and B. All the rabbits in the two groups were treated with *G. lucidum* extracts, now formulated into ophthalmic suspension. Two drops of the suspension were instilled two hourly at the start and then gradually tapered through seven days. Observed ocular changes following commencement of treatment were both photographed and recorded according to the stated grading system, both being taken systematically in an order of 1st hour, through the 12th, 24th, 48th, 72nd, 96th, 120th, 144th up to the 168th hour. Treatment was tapered following noticeable improvement (possible healing) on the ocular inflammations and infections.

Treatment of the animals in group C also commenced 24 hours following induction of ulcer and infection on the right eye cornea. A standard treatment protocol for chemical (alkaline) induced ocular injury and bacterial-induced corneal ulcers was employed. This consisted of two hourly drops of fluoroquinolone (ciprofloxacin), six hourly drops of corticosteroid (betamethasone), and eight hourly drops of atropine sulphate 1% [16, 17]. These were gradually tapered following observation of significant reductions in ocular inflammations and infections. As with the experiment groups, photo shoots and recordings according to the stated grading system were done starting after the 12th hour, through every 24 hours up to the 168th hour. While no positive active treatment was given to the rabbits in group D, in line with the treatment schedule periods of groups A, B and C, cycloplegic agent (atropine sulphate 1%) was instilled topically (1 drop), four hourly on the right eye cornea of each rabbit. Observations (photographs and recording of parameter changes) were done according to the schedules of groups A, B and C.

Data analysis

All data collected pre- and post-experiment were entered for groups A, B, C and D into a statistics software (SPSS, version 23) for analysis according to within and between groups comparison. The findings for ocular morphologic changes were entered quantitatively and coded on the grading scale of 0 to 5 based on severity [15], with the findings in all groups being presented in tables. Considering the small sample size of this experiment, alternative non-parametric tests to paired t-tests and independent t-test were used for statistical analysis of the results. All comparisons were made at $\alpha = 0.05$ (95% CI) using Wilcoxon signed-rank test for correlated (within groups) variables and Mann-Whitney U test for independent (between groups) variables.

Results

Data were collected from 16 healthy New Zealand rabbits weighing an average of 1.4 ± 0.42 kgs. Ocular parameters for various observations following treatment for 168 hours after the introduction of chemical injuries and subsequent infection with *P. aeruginosa* were coded according to the grading scale of 0 to 5 and these are presented in Tables 1 to 4. Table 1 shows the manifestations of various ocular parameters for animals in group A (experiment group 1) at the first 24th hour following injury with the chemical (1 molar sodium hydroxide), and the last 168th hour of treatment. The statistical presentation shows that although treatment with *G. lucidum* extract had a significant anti-inflammatory effect on just a few ocular structures: eyelid status change, reduced lacrimation, reduced corneal staining and edema, the clinical effect of treatment with the

extract in all ocular structures observed were highly significant, particularly with lacrimation (47%). These findings are shown in Fig. 1.

Table 1
Comparison of animals in group A at two time points – 24th and 168th hours.

	Group A									
	Time point									
	24th hour				168th hour					
Rabbit Numbers	2	8	13	15	2	8	13	15	P-value	Effect size (%)
eyelid status	3	4	4	4	1	1	2	1	0.004	30
conjunctival status	3	4	4	4	2	3	2	2	0.008	38.45
lacrimation	5	4	4	4	1	2	1	2	0.02	46.75
epithelial eruption	4	2	2	0	3	3	2	3	0.368	50
corneal staining	3	4	2	0	3	3	2	3	0.041	60
photophobia	4	2	2	2	1	3	2	2	0.19	50
discharge	5	2	2	0	1	1	1	1	0.926	3.85
corneal edema	5	5	2	5	0	2	2	3	0.008	43.75
corneal infiltrates	4	3	0	0	2	3	3	2	0.23	73.53
Wilcoxon signed-rank test @ α = 0.05										

Legend

Table 1 shows result of the effects of *G. lucidum* on the chemical-induced corneal injury when compared at two time points between the 24th and the 168th hours, following commencement of treatment with extract of *G. lucidum.*

Table 2 shows the manifestations of various ocular parameters for animals in group B (experiment group 2) at the first 24th hour following injury with 1 molar Sodium hydroxide, and subsequent infection with *P. aeruginosa* 12 hours thereafter (to induce bacterial ulcer), and at the last 168th hour of treatment with *G. lucidum*. The results show that treatment with *G. lucidum* extract had no significant difference ($p > 0.05$), in the statuses of most ocular structures after the 168th hours, except for a significant reduction in photophobia and lacrimation ($p < 0.05$). However, the clinical effect of treatment with the extract in all ocular structures observed was highly significant. The clinical effect of treatment with *G. lucidum* for bacterial infected corneal ulcers was most clinically significant (*effect size = 93%)* with a reduction of ocular discharges. This finding is also shown pictorially in Fig. 2.

Table 2
Comparison of animals in group B at two time points – 24th and 168th hours.

	Group B									
	Time point									
	24th hour				168th hour					
Rabbit Number	3	7	12	16	3	7	12	16	P-value	Effect size (%)
eyelid status	5	4	4	5	2	0	3	0	0.051	94.57
conjunctival status	5	4	4	5	3	0	3	1	0.067	79
lacrimation	5	4	5	5	3	0	3	0	0.016	6
epithelial eruption	5	5	2	2	3	2	2	3	0.607	25
corneal staining	5	5	2	2	3	2	2	3	0.607	25
photophobia	5	4	4	5	2	1	3	2	0.032	63.45
discharge	5	4	4	5	0	0	3	0	0.156	92.85
corneal edema	5	4	2	5	2	0	2	0	0.247	70
corneal infiltrates	2	5	2	2	2	1	3	1	0.198	16.67
Wilcoxon signed-rank test @ α = 0.05										

Legend

Table 2 shows result of the effects of *G. lucidum* on ulcerating corneas chemically injured using a drop of Sodium Hydroxide (NaOH) and subsequently infected with laboratory prepared *P. aeruginosa*, compared at two time points between the 24th and the 168th hours, following commencement of treatment with extract of *G. lucidum.*

Table 3 shows a comparison between manifestations of various ocular parameters for animals after 168 hours of treatments with *G. lucidum* in group B (experimental group 2) and the standard treatment protocol in group C (positive group). The results show that although treatment in group C had better clinical effects than in group B, the difference in the treatment was, however, not statistically significant ($p > 0.05$). This finding is also shown pictorially in Fig. 3.

Table 3
Comparison between animals in groups B and C after the 168th hour.

Group comparison										
Group B					Group C					
Rabbit Number	3	7	12	16	6	10	11	14	P-value	Effect size (%)
eyelid status	2	0	3	0	1	2	1	0	0.717	16.67
conjunctival status	3	0	3	1	1	1	1	1	0.763	22.75
lacrimation	3	0	3	0	2	3	3	3	0.002	48.52
epithelial eruption	3	2	2	3	0	1	1	0	0.368	50
corneal staining	3	2	2	3	0	1	1	0	0.368	50
photophobia	2	1	3	2	3	1	3	3	0.009	33.32
discharge	0	0	3	0	0	0	0	1	0.317	25
corneal edema	2	0	2	0	2	3	0	0	0.317	25
corneal infiltrates	2	1	3	1	0	0	0	0	0.867	7.15
Mann-Whitney U test @ α = 0.05										

Legend

Table 3 shows result of the comparison between the effects of *G. lucidum* (group B animals) and the standard treatment protocol (group C animals) on ulcerating corneas chemically injured using a drop of Sodium Hydroxide (NaOH) and subsequently infected with laboratory prepared *P. aeruginosa*, after 168 hours of treatment in both groups.

Table 4 shows a comparison between the manifestations of various ocular parameters for animals in group B (experiment group 2) and those in group D (placebo (negative control) group) after 168 hours of treatments with *G. lucidum* on the former (group B) and atropine alone for the latter (group D). The results show that the difference in the treatment in both groups was not statistically significant ($p > 0.05$). This finding is also shown pictorially in Fig. 4.

Table 4
Comparison between animals in groups B and D after the 168th hour.

Group comparisons										
Group B					Group D					
Rabbit Number	3	7	12	16	1	4	5	9	P-value	Effect size (%)
eyelid status	2	0	3	0	2	0	0	1	0.417	43.75
conjunctival status	3	0	3	1	2	0	0	1	0.202	80
lacrimation	3	0	3	0	3	0	0	0		
epithelial eruption	3	2	2	3	2	2	0	2	0.317	25
corneal staining	3	2	2	3	1	2	0	2	0.74	30
photophobia	2	1	3	2	0	2	3	2	0.74	30
discharge	0	0	3	0	2	0	1	0	0.607	25
corneal edema	2	0	2	0	0	0	0	0		
corneal infiltrates	2	1	3	1	1	1	0	0	0.717	16.67
Mann-Whitney U test @ α = 0.05										

Legend

Table 4 shows result of the comparison between the effects of *G. lucidum* (group B animals) and atropine sulphate alone as negative control (group D animals) on ulcerating corneas chemically injured using a drop of Sodium Hydroxide (NaOH) and subsequently infected with laboratory prepared *P. aeruginosa*, after 168 hours of treatment in both groups.

Discussion

In this study, we found a progressively improved healing effect of extract of *G. lucidum*, from the first 24th hour to the 168th (seven days of treatment). In this study, the indices of healing used to show improvement in the healing process of a purposefully induced corneal injury and then subsequently infested using *P. aureginosa* – a common, normal floral, but opportunistic microorganism – were eyelid status, conjunctival status, lacrimation, epithelial eruption, corneal staining, photophobia, ocular discharge, corneal edema, and corneal infiltrates. In group A animals (Table 1), there were observable changes in this first experimental group exposed to chemical injury alone using 1 molar sodium hydroxide. Our finding showed that although treatment with *G. lucidum* extract had a significant anti-inflammatory effect on just a few ocular structures: eyelid status change, reduced lacrimation, reduced corneal staining and edema, the clinical effect of treatment with the extract in all ocular structures observed were highly significant, particularly with lacrimation (47%). These findings are shown in Fig. 1. As shown, both the clinical effect and the observable signs demonstrates that treatment with *G. lucidum* after the 168th hour significantly improves the corneal healing process following a chemical (alkaline) injury. However, as can be seen from Fig. 1, and as is typical with most chemical injuries, specifically from sodium hydroxide that causes corneal damage through pH change, ulceration, proteolyzes and collagen synthesis

defects to the cornea, healing following chemical injuries takes time, way longer than 7 days to achieve both significant physically observable signs of healing [18]. This time limitation was a key challenge in this study. Still, and as noted by Singh [19], chemical injuries, particularly those of alkaline origin, to the cornea are potentially blinding ocular injuries and constitute a true ocular emergency requiring immediate assessment and initiation of treatment [19]. Our finding demonstrates that instituting treatment with extract of *G. lucidum* as early as within the first 24th hour of occurrence has potential to prevent extensive and penetrating damage to the cornea, and other ocular surface tissues that present a risk to irreversible vision loss.

Furthermore, among animals in group B (experiment group 2) as shown in Table 2, our finding demonstrates that treatment with *G. lucidum* extract did not significantly change the ocular morphology of most of the animals after the 168th hours, except for a significant reduction in photophobia and lacrimation. Our findings showed that the differences in the analysis of the quantitative morphological variables observed between the manifestations of various ocular parameters in the animals of this group at the first 24th hour following injury with 1 molar Sodium hydroxide, and subsequent infection with *P. aeruginosa* 12 hours thereafter (to induce bacterial ulcer), and at the last 168th hour of treatment with *G. lucidum*, was not significant. Nonetheless, the converse was true for the clinical effect of treatment with the extract of *G. lucidum*. As shown in Table 2, in all ocular structures observed the differences in the morphological changes between the 24th and the 168th hours were clinically significant (*effect size > 5%)*. This finding, therefore, showed that the clinical effect of treatment with *G. lucidum* for bacterial infected corneal ulceration is significant. The clinically significant effect (*effect size = 93%*) was found to be most effective with the reduction of ocular discharges – a good morphological indication of a reduction in microbial load [13]. This finding is also shown pictorially in Fig. 2, thus confirming findings of previous studies [5, 11, 12], that the antibacterial effect of *G. lucidum* on non-ocular related inflammatory injuries and infective ulcers in other parts of the human body is clinically effective. In addition, our finding demonstrates that *G. lucidum* has proven to be effective in managing bacterial infections that have shown resistance [13], as well as shown to be an effective alternative to treating bacterial infections and inflammatory injuries to the human body [14], may as well be an effective alternative to the management of inflammatory and ulcerative conditions of the corneal and other tissues of the ocular adnexa.

Going forward, our study compared the treatment effect of extract of *G. lucidum* with existing standard treatment protocol for chemical (alkaline) induced ocular injury and bacterial induced corneal ulcers consisting of two hourly drops of fluoroquinolone (ciprofloxacin), six hourly drops of corticosteroid (betamethasone), and eight hourly drops of atropine [16, 17]. We found that after the 168th hour of treatments with *G. lucidum* on animals in group B (experimental group 2) and treatment with the standard treatment protocol on animals in group C (positive control group), treatment in group C had better clinical effects than in group B (*effect size > 5%)*, as shown in Table 3. However, as can be seen from Table 3, the difference between the two treatment protocols was not statistically significant ($p > 0.05$). This finding is also shown pictorially in Fig. 3. The implication of this finding, therefore, is a demonstration of the fact that treatment with extract of *G. lucidum* may be an effective alternative to the treatment of bacterial induced corneal ulcerative and inflammatory injuries. This further justifies the finding of infective and inflammatory injuries to other non-ocular tissues of the human body [14].

Finally, to further demonstrate the healing effects of extracts of *G. lucidum* on the cornea and other ocular adnexa, we compared the morphological changes after 168th hour between animals in group B (experiment

group 2) and those in group D (placebo – negative control group). As shown in Table 4, we found that after 168 hours of treatments with *G. lucidum* in group B, and treatment with atropine only on animals in group D, there was no statistically significant difference ($p > 0.05$), observed in both groups. However, our finding further showed a clinically significant effect, in the healing observed in group D animals over those group B animals (Fig. 4). This was an interesting finding of our study that the animals in group D (negative control) that were administered with atropine sulphate alone showed better clinical improvement than those treated with *G. lucidum*. Atropine sulphate was purposively administered to control ciliary spasms in the eyes of the animals in the negative control group. It was interesting to observe that atropine (primarily a cycloplegic agent) also known to show anti-inflammatory secondary effects [20, 21], resulted in significantly better healing effects compared with *G. lucidum*. This finding may explain the importance of introducing an anti-inflammatory treatment protocol as early as possible in the management of both chemical and bacterial induced corneal ulcerations.

To summarize, therefore, we note that while there was limited empirical data in literature on the ocular-related healing effects of *G. lucidum* to compare our findings with, we found extracts of the mushroom to show an observable reduction in the severity of changes in the ocular parameters affected following induction of chemical injury and subsequent bacterial corneal ulceration. Specifically, we note a significant reduction in eyelid swelling and lacrimation in animals treated with *G. lucidum* after the 168th hour of treatment. This is evident with the animals in groups A and B, where the eyelid and conjunctival severity were significantly reduced progressively (Figs. 1 and 2). Although no significant change was noticeable in a few (epithelial eruption, epithelial staining, corneal fluorescein staining, and corneal edema), we found the clinical healing effects of *G. lucidum* extracts to be highly significant, particularly for the reductions observed with ocular discharges, photophobia and corneal infiltrations. This we found, while not being consistent with the healing effect observed in a study on paw edema following intra-muscular instillation of *G. lucidum* [22], was nonetheless consistent with the healing effect observed in another study on gastric ulcers [23]. Other studies that compared the clinical healing effects of extracts of *G. lucidum* with commonly used antibiotics, such as gentamycin sulphate [13] and fluoroquinolones [20], found it to be equally effective in other body structures. We found this not to be the case with ocular healing effects, as standard treatment with a combination of fluoroquinolone, corticosteroid and atropine sulphate, and even with using atropine sulphate alone, turned out to show better clinical effect than with treatment with *G. lucidum* alone.

Conclusion

Extracts of *G. lucidum* have shown the potential to be an alternative treatment approach for chemical (alkaline) and bacterial (*P. aeruginosa*) induced corneal ulceration. The healing (anti-inflammatory and anti-infective) effects of *G. lucidum* have shown clinically significant healing effects after 168 hours of treatment compared with that of 24 hours. Although corneas treated with standard protocol showed better clinical effects than *G. lucidum*, the current investigation has shown the importance of the early institution of the anti-infective and anti-inflammatory protocol in the management of corneal ulcers. Therefore, *G. lucidum*, where available, may be a useful anti-infective alternative for chemical-induced and bacterial-infected corneal ulcerative condition. In this regard, therefore, our study notes that despite the limitation of time, *G. lucidum* has the potential to be used as an alternative treatment approach for chemical-induced and bacterial-infected corneal ulcerations in resource-constrained settings. Hence, we recommend further research to explore the anti-inflammatory potentials of *G. lucidum* in population-controlled settings.

Declarations

Ethics Approval

Approval for this study was obtained from the Institutional Ethics Review Committee of Masinde Muliro University of Science and Technology (MMUST, IERC) (MMU/COR: 403009 (VOL. 1). All ethical guidelines, consistent with the Prevention of cruelty to Animals Act of Kenya [24, 25], and the Committee on Animal Research and Ethics' guideline for ethical conduct in the care and use of animals, including responsible and ethical disposal of animals during clinical and laboratory research [26], were strictly adhered to in this study.

Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and analyzed during this study can be made available from the corresponding author upon reasonable request.

Competing interests

The authors declare no financial or competing interest in the research conducted and subsequent writing of this paper.

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Authors' contribution

EOV was involved in the study design, data collection, data analysis, drafting and reviewing of the manuscript. AKM was involved in the study design, data collection, data analysis, drafting and reviewing of the manuscript. POO was involved in data collection, drafting and reviewing the manuscript. AVD was involved in drafting and reviewing the manuscript to its final stage. KPM was involved in drafting and significant review of the manuscript to its final stage. All authors have read and approved the final version of the manuscript.

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Figures

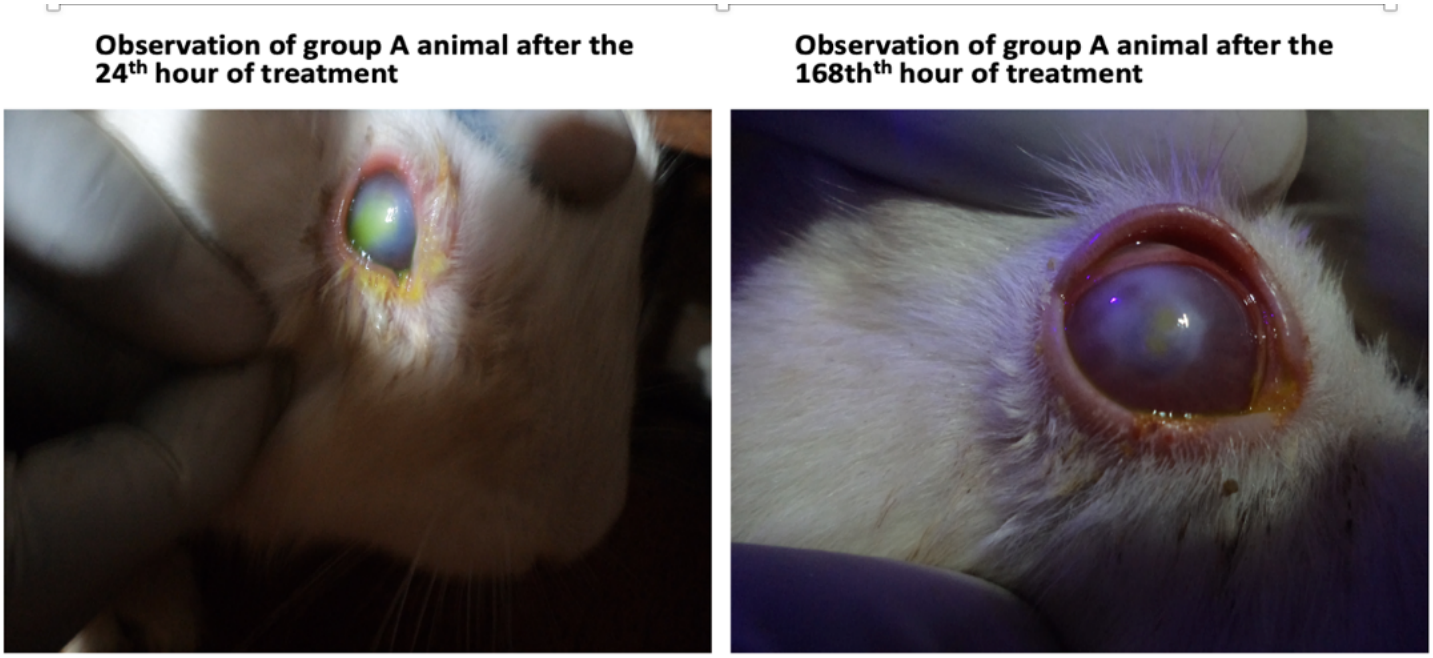

Figure 1

Presentation of the effect of *G. lucidum* on the chemical-induced corneal injury on group A animals after 168th hour of treatment. As shown, animals in group A (*right)* appeared to have healed, with fewer discharges, lacrimation and better corneal epithelial changes, after 168 hours of treatment with *G. lucidum.*

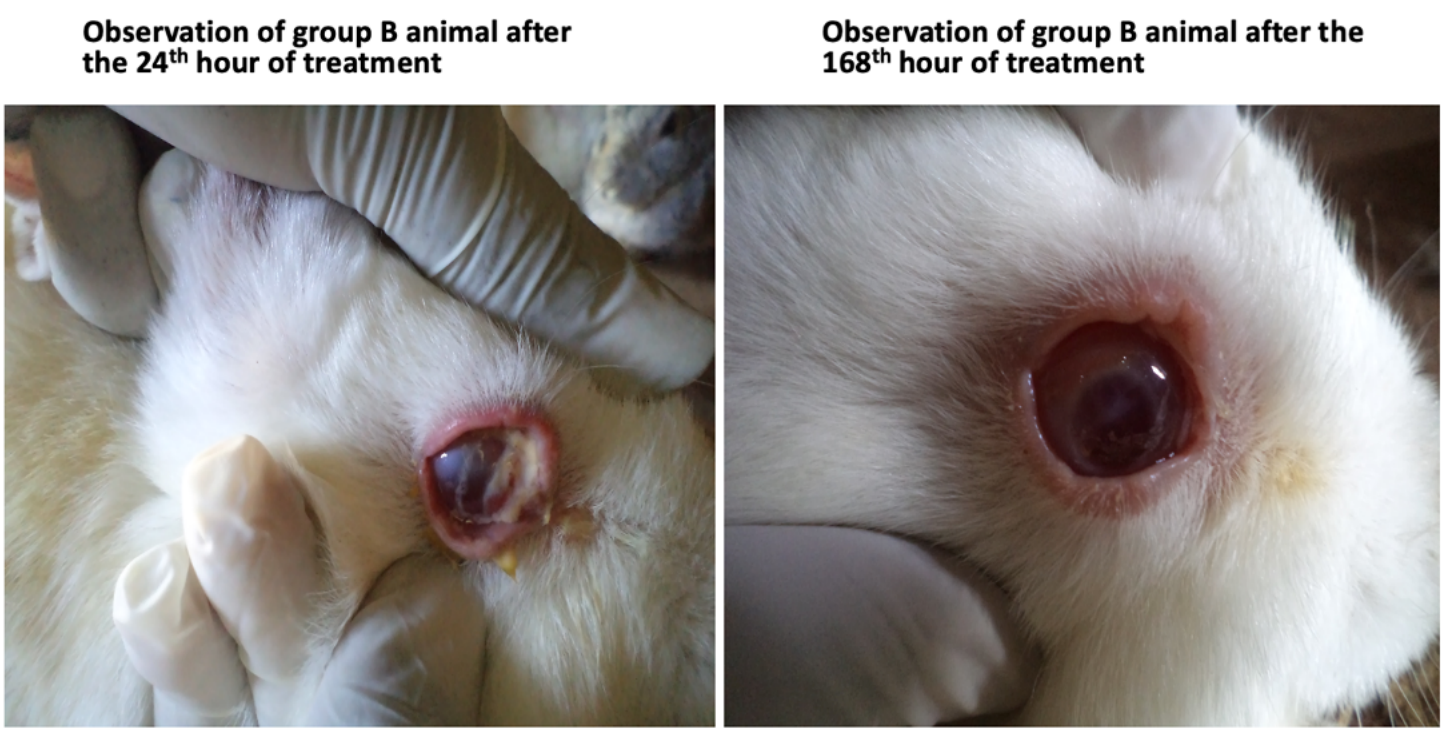

Figure 2

Presentation of the effect of *G. lucidum* on chemical-induced corneal injury and subsequently infected with *Pseudomonas aeruginosa* on group B animals after 168th hour of treatment. As shown, animals in group B (*right)* appeared to have healed better, with fewer discharges, lacrimation and better corneal epithelial changes, after 168 hours of treatment with *G. lucidum.*

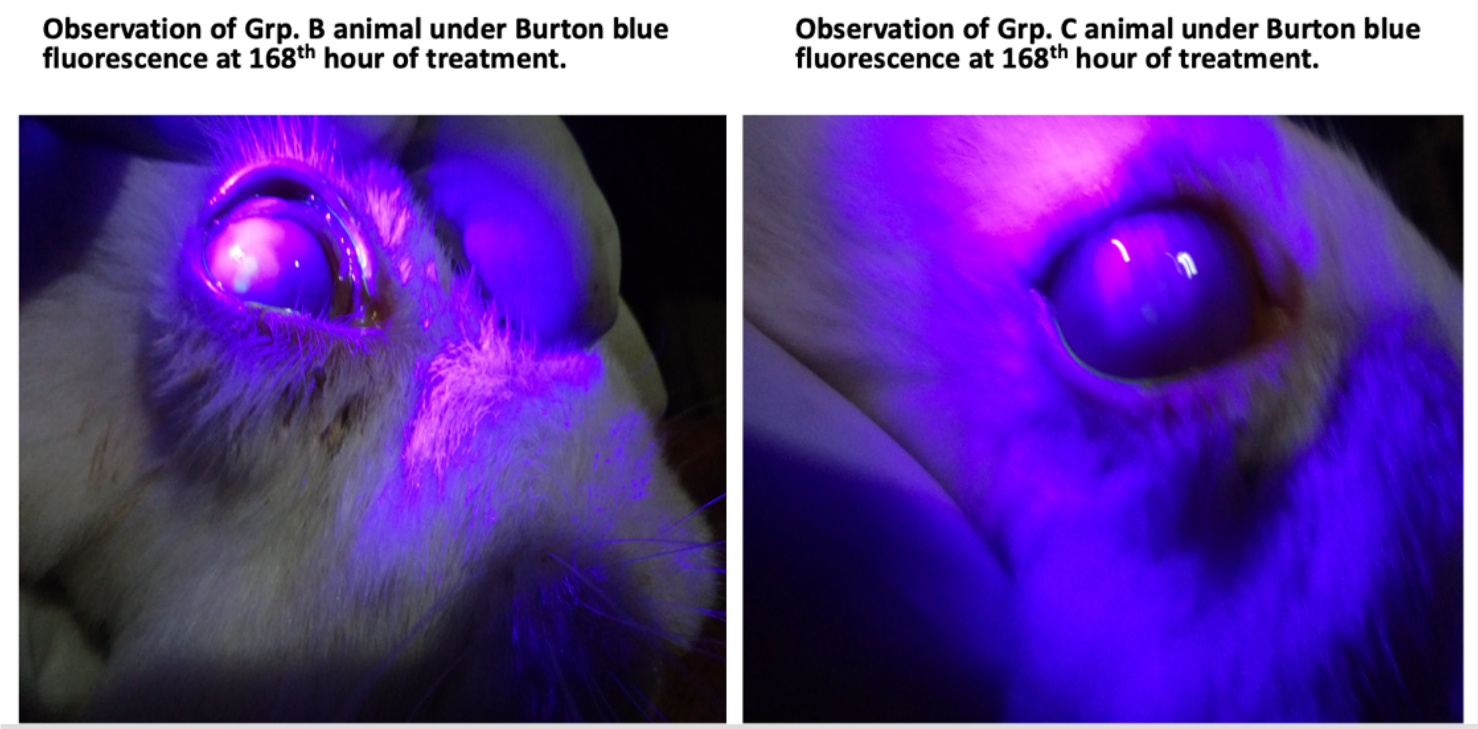

Figure 3

Presentation comparing the effect of *G. lucidum* on chemical-induced corneal injury and subsequently infected with *Pseudomonas aeruginosa* (group B animals), with control group C animals *(right),* treated with standard treatment protocol after 168th hour. As shown, animals in group C (*right)* did not physically appear (observation on burton blue lighting) to have healed better than group B animal *(left),* after 168 hours of treatment with *G. lucidum.*

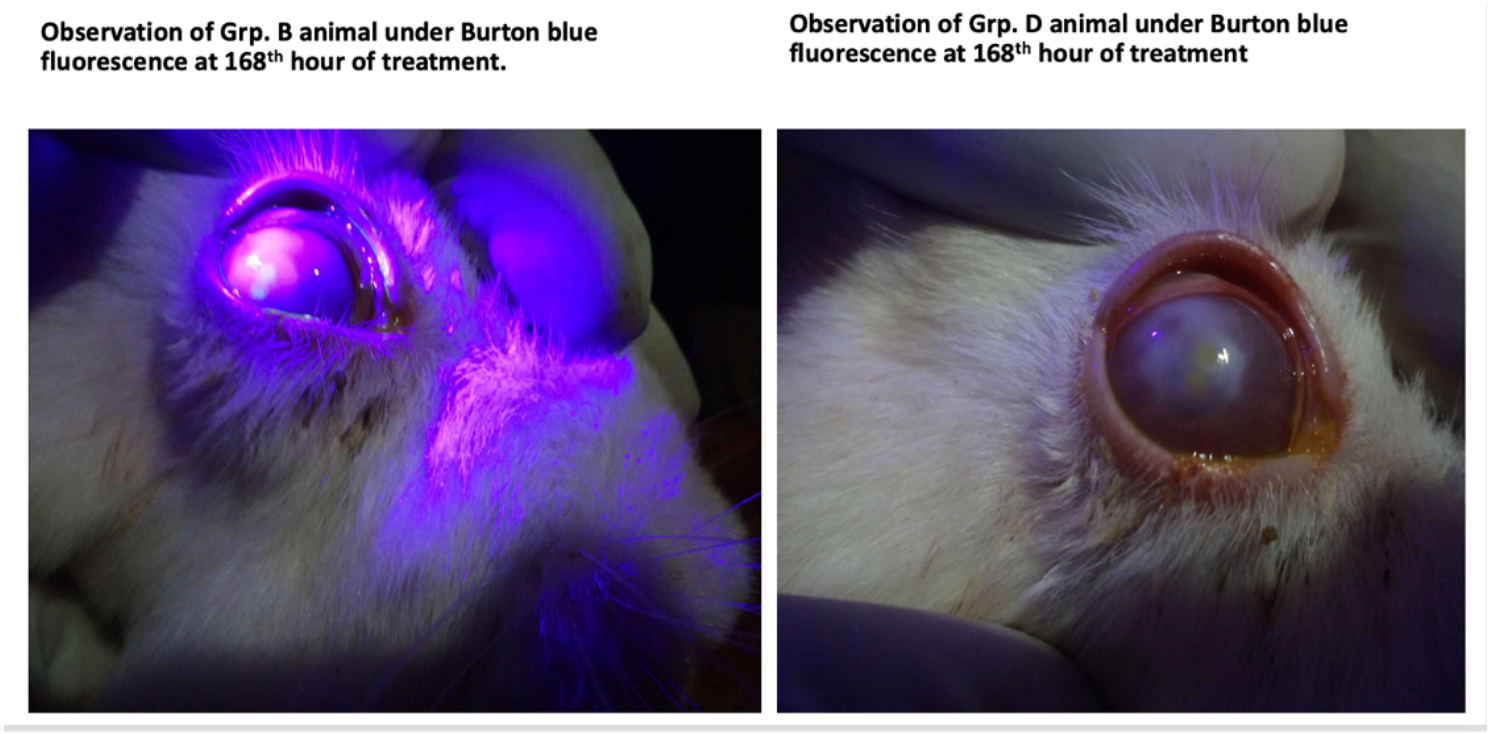

Figure 4

Presentation comparing the effect of *G. lucidum* on chemical-induced corneal injury and subsequently infected with *Pseudomonas aeruginosa* (group B animals), with control group D animals (placebo group) after 168th hour. As shown, Group D animals *(right)* appeared to have healed better.