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Curative and Protective Effects of Penicillin G on Experimental *Chlorophyllum molybdites* Poisoning in Mice

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Abstract: The aim of this study is evaluate the curative and protective effects of penicillin G in mice poisoned with the lyophilized extract of *Chlorophyllum molybdites*. Fifty Swiss albino mice were divided into 5 groups of 10 mice each. Mice in group 1 were pretreated with penicillin G at 38, 280 IU kg^{-1}, i.p. and then dosed with LD_{99} of *C. molybdites* (741 mg kg^{-1}) i.p., mice in group 2 were dosed with the extract and then treated with penicillin G, while mice in group 3 were dosed with the extract only. Mice in groups 4 and 5 were dosed with penicillin G and physiological saline solution, respectively. The mice were monitored for clinical signs of toxicity, pathological lesions and death over a period of 72 h. The mean time of death in mice from penicillin-treated groups 1 and 2 were compared with those in the extract-treated group using one-way analysis of variance (ANOVA) and values of $p<0.05$ were considered significant. The result showed a significant reduction in the severity of clinical signs and mortality in penicillin-treated groups 1 and 2 compared to the group dosed with only the extract. There was a significant difference in the mean time of death in mice from groups 1, 2 and 3. However, there was no reduction in the severity of lesions in mice from groups 1 and 2 treated with penicillin G compared with extract-treated group. Therefore, this study has shown that penicillin G has significant curative and protective effects in mice poisoned with the lyophilized extract of *C. molybdites*. This result may prove useful in the treatment of humans and animals suffering from *C. molybdites* poisoning.

Key words: *Chlorophyllum molybdites*, penicillin G, treatment, prophylaxis

INTRODUCTION

Reports of severe and fatal mushroom poisonings have increased worldwide (Diaz, 2005) as it continues to be a problem faced by health care professionals (Fischbein *et al*., 2003). In USA, combined data of the American Association of Poison Control Center (AAPCC) and mushroom poisoning registry of the North American Mycological Society shows that approximately 5 patient exposures to toxic mushrooms per 100,000 population occur per year (Trestrail, 1991). However, records involving cases of mushroom poisoning are incomplete and unreliable, especially in the developing countries where poisoning mostly occur in rural settings without competent clinics and clinicians.

Chlorophyllum molybdites enjoys worldwide distribution as one of the most common lawn mushrooms in both urban and rural areas, which helps to account for its many human encounters. In 1989, data from AAPC showed that *C. molybdites* poisoning represented about 23% of all reported

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cases of mushroom poisoning in USA (Trestrail, 1991). However, this mushroom has been associated with limited fatalities in man, as only one death in a child has been attributed to *C. molybdites* poisoning in literature (Chestnut, 1900); although the morbidity and course of the disease may be prolonged, largely limited to severe gastrointestinal problems (Stenklyt and Augenstein, 1990).

Treatment of mushroom poisoning continues to pose serious challenges to scientists and medical/veterinary practitioners around the world. In most instances, there is no antidote to mushroom poisoning and most victims are treated only symptomatically, with some ending fatally. However, some agents such as penicillin G have shown good promise in the treatment of amatoxin poisoning in man (Faustich and Zilker, 1994). Therefore, the objective of this study is to evaluate the curative and protective effects of penicillin G in mice dosed intraperitoneally with the lyophilized extract of *C. molybdites*.

MATERIALS AND METHODS

Mushroom Collection, Identification and Preparation

Chlorophyllum molybdites was collected from the wild in Zaria, Nigeria and taxonomic identification was made at the Department of Botany and Microbiology, University of Ibadan, Nigeria. The mushroom was air-dried for 4 h and subsequently oven-dried at a temperature of 45°C over 72 h. The dried mushroom was powdered and 100 g of it was macerated with 1 L of distilled water for 24 h. The resultant extract was filtered and the filtrate lyophilized to an amorphous substance referred to as the extract. Appropriate stock dilution of the extract was prepared in physiological saline solution for this study.

Experimental Animals

Locally bred Swiss albino mice were housed under standard condition in the laboratory animal room of the Department of Veterinary Physiology and Pharmacology, Ahmadu Bello University, Zaria, Nigeria. The animals were fed on standard mice pellets, while water was provided *ad libitum*. The experiment was conducted under the supervision of the Animal Welfare and Ethics Committee of the Ahmadu Bello University, Zaria and in accordance with the international protocol on animal welfare.

Evaluation of the Curative and Protective Effects of Penicillin G on Experimental *C. molybdites* Poisoning in Mice

Fifty Swiss albino mice, 4-6 weeks old and weighing 21-28 g were divided at random into 5 groups of 10 mice each. All the mice in groups administered with the extract were dosed at the previously determined LD_{99}~741 mg kg^{-1}, while those given penicillin G were dosed at 38,280 IU kg^{-1} i.p., which was arrived at after a series of pilot study. The mice in group 1 were pre-treated with penicillin G i.p., followed by the LD_{99} of the reconstituted extract i.p., 10 min later (to evaluate protective effect of penicillin G). Mice in group 2 were administered with the LD_{99} of the extract, followed by penicillin G at the onset toxic signs 10 min later (to evaluate curative effect of penicillin G), while the mice in group 3 were given only the LD_{99} of the extract ip. Animals in groups 4 and 5 were dosed with only penicillin G (38, 280 IU kg^{-1}) and physiological saline solution (1 mL kg^{-1}) i.p., respectively.

The animals were examined for signs of toxicity and postmortem gross examinations conducted on all dead mice during the study and those euthanized at the end of the study. Histopathological processing and examinations were conducted on sections of the tissues of the brain, liver, spleen, intestines, stomach, kidneys, heart and lungs using standard procedures (Luna, 1962). The average time of death expressed as mean±SEM over a period of 72 h was recorded. Data obtained in all the groups were analysed using one-way analysis of variance (ANOVA) and values of $p<0.05$ was considered to be statistically significant.

RESULTS

Clinical signs of toxicity observed in mice from group 1 included prolonged depression and mild increase in the rate and force of abdominal contraction. Death occurred in three mice within the 72 h period of observation. The average time of death was 261.0±48.2 min. The remaining 7 mice survived beyond the 72 h period of observation. Toxic signs observed in mice from group 2 included abdominal contraction and prolong depression. Death was observed in 3 mice with the mean time of death of 440.0±18.4 min, while the remaining 7 mice in this group survived beyond the period of observation. Signs observed in mice from group 3 included prolonged depression, abdominal contraction and anorexia, with death occurring in all the mice within the 72 h period of observation. Marked excitement, convulsion and respiratory distress, which preceded death of all the mice in this group, were not observed in those mice that died in the penicillin-treated groups 1 and 2. The average time of death was 248.2±28.3 min. All the mice in groups 4 and 5 survived beyond the 72 h period of observation without any apparent sign of toxicity. There was a significant difference ($p<0.05$) in the mean time of death in mice from all the groups (Table 1).

The post mortem gross lesions observed in mice in group 1-3 included enlarged and congested liver, congested kidneys and spleen, catarrhal enteritis and congestion of brain capillaries. There were no apparent post mortem gross findings in mice in groups 4 and 5. Histopathologically, mice in groups 1, 2 and 3 showed congestion and necrosis of the liver and disruption of the hepatic architecture (Fig. 1) congestion and necrosis of heart, intestine and renal tubules of the kidneys with mononuclear

Table 1: Curative and protective effects of penicillin G (38,280 IU kg^{-1}., i.p.) on experimental poisoning of mice by lyophilized extract of *Chlorophyllum molybdites* (741 mg kg^{-1} b.w. i.p.)

				Mice		
		Treatment design		No. of survived/		Mean period
Groups	No. of mice	1st treatment	2nd treatment	No. of dosed (within 72 h)	Survival (%)	of survival (min)
1	10	Penicillin G	Extract		70	261.0±48.2
2	10	Extract	Penicillin G	7/10	70	440.0±18.4
3	10	Extract only	-	0/10	00	248.2±28.3
4	10	Penicillin G only	-	10/10	100	-
5	10	Physiological saline solution	-	10/10	100	-

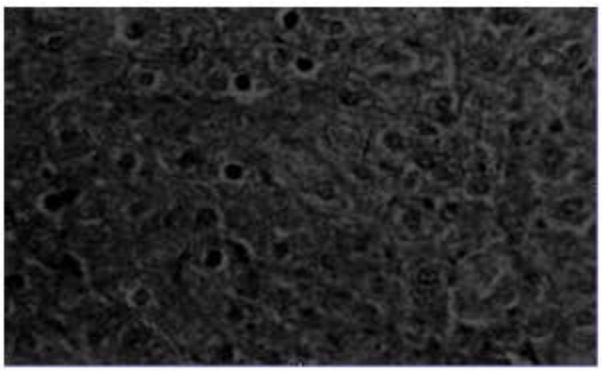

Fig. 1: Liver of mice pretreated with penicillin G showing disorganized hepatic architecture and diffuse necrosis of the hepatocytes. H and E×400

cell infiltration, but no apparent lesion was observed from mice in groups 4 and 5. This result showed no apparent difference in the degree of severity of lesions at both gross and histopathological examinations between those few mice that died in penicillin G treated groups when compared to those given the mushroom extract only.

DISCUSSION

Most animal experiments involving evaluation of antidotal, curative or protective effects of agents to combat mushroom poisoning used rats and mice as the experimental subjects (Floerscheim *et al*., 1971; Floerscheim, 1976; Choppin and Desplaces, 1978). However, this group of animals do not absorb the mushroom toxins from the gastrointestinal tract, which is the route of exposure in man. Studies have however revealed that lesions similar to those produced from oral poisoning in humans can be obtained, if the mushroom is administered parenterally in rats and mice (Choppin and Desplaces, 1978; Parish and Doering, 1986).

In the present study, administration of penicillin G was shown to have significant prophylactic and therapeutic effect in mice dosed with the lyophilized extract of *C. molybdites*. In addition, the drug was shown to reduce the severity of the clinical signs, as excitement, respiratory distress and convulsion observed in mice administered with only the mushroom extract were not observed in the penicillin G-treated groups. A significant increase in the mean time of death in the penicillin G-treated groups compared to the group treated with only the extract further confirmed the efficacy of penicillin G in the management of *C. molybdites* poisoning.

Penicillin G as shown by this study, however, did not confer significant protection from injury induced by the mushroom toxins on tissue and organs in the few mice that died from penicillin G-treated groups, when compared to group treated with the extract only. The reason for this was not clear but may relate to the frequency of dosing with penicillin G. It is possible that increasing the dose and frequency of administration of penicillin G may likely improve its protective effect on tissue injury. In man, penicillin G is administered repeatedly and at a very high dose in the management of amatoxin poisoning. However, caution should be exercised in the choice of dosage since penicillin G at a very high dose in man is known to induce cerebral convulsion, hepatic encephalopathy and allergic shock leading to clotting dysfunction and pseudomembraneous enterocolitis (Faulstich and Zilker, 1994).

Although the present study did not focus on the mechanism responsible for the protective and curative effect of penicillin G in *C. molybdites* poisoning, the drug is known to block the transport system responsible for amatoxin uptake by the hepatocytes (Spoerke, 2001).This mechanism may also be responsible for the protective and curative effects of penicillin G observed in this study. It is however possible that some other mechanisms are involved. Therefore, there is the need to further investigate the mechanism responsible for the effect of penicillin G in *C. molybdites* poisoning in mice as observed in this study.

In conclusion, this study for the first time has demonstrated the protective and curative effect of penicillin G in mice poisoned with the lyophilized extract of *C. molybdites*. In addition, penicillin G was shown to reduce the severity of toxic signs but did not completely abolish them. However, treatment with penicillin G did not reduce the severity of the lesion. It is recommended that further work should be done on larger monogastric animals especially dogs using the oral route of ingestion. The result of this will serve as the basis of using penicillin G in the treatment of *C. molybdites* poisoning in man.

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