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Assessing the Cytotoxic Effects of the Anti-HIV Drug, Garani MW1

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Abstract

The use of herbal medicine for the cure of HIV and AIDS is reported to be on the rise. Most of these herbal medicines have not been subjected to rigorous clinical testing and thus are considered unsafe within the medical establishment. The objective of the present study was to assess the cytotoxic potential of Garani MW1 aqueous extract using the brine shrimp assay. Brine shrimp larvae were incubated in Garani MW1 aqueous solution of 0, 100, 400, 600, 800,

and 1000 μ L v/v for 12 and 24 hours. It was observed that Garani MW1 aqueous solution killed brine shrimps larvae in a dose and time dependent manner. Our results indicate that the herbal concoction has cytotoxic effects at higher concentrations of above 400 μ L v/v.

Keywords: Brine shrimp, cytotoxicity, herbal medicine, Garani MW1

Introduction

Many HIV/AIDS patients around the world are using therapeutic herbs to treat this disease. Unfortunately, most of these herbs that are used have not been clinically tested to certify their safety and efficacy, even though anecdotal evidence may support their use.

In Africa, in the absence of adequate biomedical services it is estimated that 80-85% of the people make use of traditional healers' services[1]. Traditional healers in rural African communities tend to be the first professionals consulted by people with health problems because they are easily accessible geographically and provide culturally accepted treatments. Moreover, they are relatively affordable when compared to hospitals [2] and have credibility, acceptance and respect among the population they serve [3].

In recent years in Malawi, there have been many claims from traditional healers that they have discovered herbal medicines for HIV/AIDS cure. One of the most advertised herbal medicine is called Garani MW1. Since its discovery in 2007 as a drug that cures HIV and AIDS, Garani MW1 has received both positive and negative sentiments. There have been numerous articles in the media quoting people, especially patients, giving testimonies about the wonders that this herbal concoction has done to their ailments. On the contrary, the scientific world seems to brush aside the claims of the potency of this drug even though no scientific tests have been done to disprove its efficacy. An article on Africacheck.org^[3] dismissed the claims that Garani MW1 cures HIV and Aids and numerous other diseases and ailments as untrue, irresponsible and that the claims should be condemned.

Screening for biologically active natural products requires the right bioassays. Detection of compounds with the desired activity in complex plant extracts depends on the reliability and

sensitivity of the test systems used [4]. This study is aimed at evaluating the aqueous extracts of Garani MW1 for its cytotoxicity effects using the brine shrimp assay.

Materials and methods

Plant material

Packaged powdered Garani MW1 was anonymously purchased from the drug distributor in Blantyre City, Malawi.

Preparation of extract

The aqueous extract was prepared by making a suspension of 100g of the powder in 500 mL of distilled water. The suspension was left to stand overnight at room temperature, with constant agitation. The mixture was filtered twice, first using ordinary filter paper and then Whatman-41 filter paper.

Brine shrimp toxicity assay

For brine shrimp assay, the procedure described by Meyer et al.[5] was followed. In brief, a rectangular dish (22 x 32 cm) was compartmentalized into two unequal halves with plastic divider of 2 mm with several holes and filled with artificial seawater (28 g sea salt/L, Sigma). Approximately 25 mg eggs (*Artemia salina* Sera, Heidelberg, Germany) were sprinkled in the larger compartment, which was darkened, while the smaller compartment was illuminated. After 24 hours, phototropic nauplii (brine shrimp larvae) were collected by pipette from the lightened side. A 1mL volume of 0, 100, 400, 600, 800 and 1000 μ L of the extract prepared in artificial sea water was placed in labeled eppendorfs. In each eppendorf 10 shrimp larvae were transferred. The eppendorfs were placed under the illumination at room temp (25 to 28 degrees Celsius), and the numbers of survivors were counted after 12 and 24 hours.

Statistical analysis

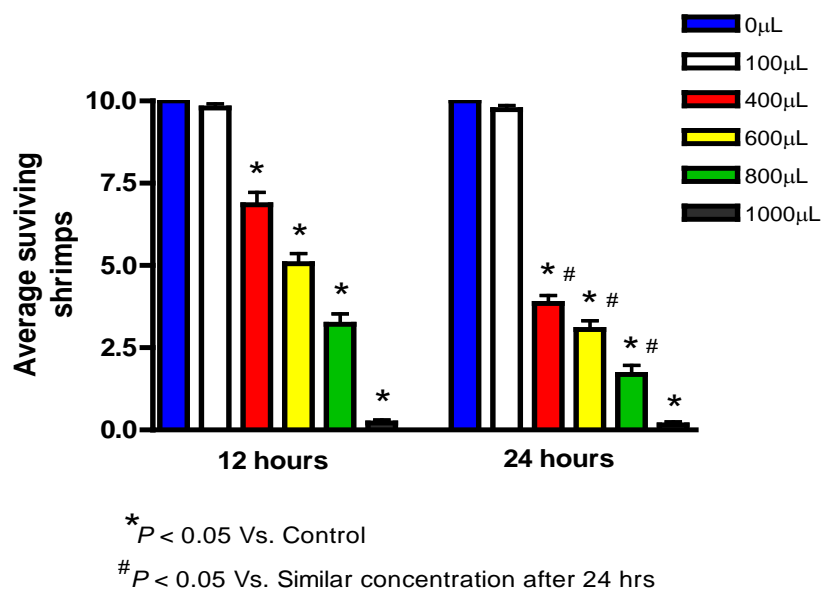
The results were analyzed on the Prism 4 statistical program (GraphPad, San Diego, CA,

USA). All data are expressed as mean \pm SEM. Data were tested for normality with the Kolmogorov–Smirnov test. One-way ANOVA (with Bonferroni *post hoc* test if $P < 0.05$) was used for statistical analysis. Differences were regarded statistically significant if $P < 0.05$.

Results and discussion

The figure shows that after 12 hours of incubation of brine shrimp larvae in different concentrations of Garani MW1 aqueous solution there was increased death of the shrimp larvae in a dose dependent manner. The increase in the deaths of the brine shrimp larvae was statistical significant when compared to the control from the concentration of 400 μ L upwards ($p < 0.05$). After 24 hours of incubation a similar trend of events as after 12 hours were observed and the number of deaths was even more significant when comparing the concentrations starting from 400 μ L versus the control ($p < 0.05$). Comparing the similar concentrations of 400 μ L, 600 μ L, and 800 μ L at 12 hours and 24 hours, there were significantly more dead shrimp larvae at 24 hours versus 12 hours ($p < 0.05$).

In recent years, herbal medicines have received great deal of interest as an alternative to clinical therapy, and the demand to these therapies are rapidly increasing. The scarcity of scientific evidence on the safety of herbal medicines has raised concerns regarding the toxicity and detrimental effects of these remedies [6]. This study reports the safety evaluation of Garani MW1. The study used different concentrations of Garani MW1 extract prepared in distilled water. This herbal mixture has been purported to cure several ailments including HIV and AIDS. Cytotoxic assay using brine shrimp larvae assay was carried out according to the method by Meyer et al. [5]. Our preliminary results indicate that Garani MW1 has cytotoxic effects at higher concentration of above 400 μ L v/v. These cytotoxic effects are dose and time dependent. This study presents valuable data on the cytotoxicity of Garani MW1 aqueous extract at higher concentrations, which could be very useful for future *in vivo* or clinical study of Garani MW1. However, further toxicity studies are needed.



Effects of different concentrations of Garani MW1 aqueous solution to brine shrimp larvae mortality after 12 and 24 hours of incubation.

References

[1] UNAIDS (2006) Collaborating with traditional healers for HIV prevention and care in sub-Saharan Africa: suggestions for programme managers and field workers. Geneva: UNAIDS.

[2] Peltzer, K and Mngqundaniso, N (2008) Patients consulting traditional health practitioners in the context of HIV/AIDS in urban areas in Kwazulu-Natal, South Africa. African Journal of Traditional, Complementary and Alternative Medicines. 5, 370-379.

[3] <http://africacheck.org/reports/claim-that-malawi-wonder-herb-cures-hiv-and-aids-is-untrue-and-irresponsible/>.

[4] Hussain, A., Zia, M and Mizra, B (2007) Cytotoxic and antitumor potential of Fagonia cretica L. Turk J Biol. 31, 19-24.

[5] Meyer, BN, Ferrigni, NR and Putman, JE (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45, 31-34.

[6] Saad, B., Azaizeh, H., Abu-Hijleh and G; Said, O (2006) Safety of traditional Arab herbal medicine. Evid Based Complement Alternat. Med. 3, 433-439.

Authors Column



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Dr. Lampiao is a reproductive physiologist and his research interests are on reactive oxygen species in human spermatozoa and how they affect fertilizing capability; effect of insulin, leptin and cytokines on human sperm function; and the influence of herbal extracts on reproductive function.