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Acute and Reproductive Toxicity of Freeze-dried and Steam-dried Extracts of Tradomal® in *Drosophila melanogaster*

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ABSTRACT

Background and Purpose: Tradomal® is an herbal preparation containing *Senna siamea* that is marketed for the treatment of malaria in Northern Nigeria. The study evaluated the acute and reproductive toxicity of freeze-dried and steam-dried extracts of Tradomal® in *Drosophila melanogaster*.

Methods: The acute toxic effect of the plant was evaluated by determining its LC_{50}. The reproductive toxicity of the extracts was evaluated via its effect on the eclosion of adult melanogaster flies.

Results: Both steam-dried and freeze-dried extracts of Tradomal® showed LC_{50} of 2767.83 mg/10 g and 4475.74 mg/10 g of meal respectively. Although the steam-dried extract did not affect fly eclosion, the freeze-dried extract significantly ($P<0.05$) inhibited the emergence of adult flies from the pupae.

Conclusion: The extracts are relatively safe, but the method of drying can influence their effects on reproduction in fruit flies.

INTRODUCTION

Most of the inhabitants of low- and middle-income countries rely on the use of herbal medicines for their immediate and long-term health care needs. Interestingly, some developed countries such as China, the USA, and the UK have also embraced the use of herbal remedies under the umbrella of complementary medicine, to provide their citizens with healthcare needs. This global upsurge in the use of herbal medicines is not unrelated to their availability, relative affordability, and perceived safety (Merlin *et al.*, 2019). Concern about the safety of herbal preparations is growing increasingly, perhaps because many plants used in traditional medicine or as food have demonstrated some toxic activities (Kuete, 2014). This, in addition to the reality that regulation of herbal remedies in Nigeria is not well established, makes it imperative to examine the safety profile of commonly sold herbal preparations like Tradomal®.

Tradomal® is a brand of herbal antimalarial medicine, produced by Tradomed Pharmaceuticals, Kaduna State, Nigeria. The herbal medicinal product is formulated in the form of teabags containing 100% *Senna siamea* as inscribed on the pack. *Senna siamea* is an evergreen tree, often between 15-20 cm tall, with a straight trunk up to 30 cm in diameter; bole short, crown usually dense and rounded at first, later becoming irregular and spreading with dropping branches; bark grey or light brown, smooth but becoming slightly fissured with age (*Lose et al*., 2005). It is commonly called yellow cassia; Bombay blackwood, Cassod tree, ironwood, Kassaof tree, pheasant wood (in English); cassia amarilla, cassia de Siam, flamboyant Amarillo (in Spanish); bois perdrix and cassia (in French). The décoction of *S. siamea* has been used locally as an antimalarial (Lose *et al.*, 2000).

The methods of preparing plant extracts can affect their phytochemical compositions, which are known to influence safety and efficacy. The aqueous extract of *S. siamea* was dried using both freeze-drying and steam-drying methods. The effect of these two extracts on the mortality and eclosion of *Drosophila melanogaster* was examined.

MATERIALS AND METHODS

Materials

Tradomal® (Batch No: 001; Manufacturing date: September 2018; Expiry date: October 2020) was obtained from Tradomed Pharmaceuticals, Kaduna State, Nigeria. Chemicals used for the study included ferric chloride solution, Dragendorff's solution, Molisch's reagent, sodium hydroxide solution, concentrated sulfuric acid solution, chloroform, ammonia solution, and magnesium chips. Distilled water, agar agar, baking yeast, nipagin, cornmeal flour, and ethanol were also used. All reagent solutions were freshly prepared before use.

The active *Drosophila melanogaster* flies were obtained from the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), Jos, Plateau State, Nigeria, and bred in the fly room of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

Methods

Phytochemical Screening

The presence of carbohydrates, alkaloids, anthraquinone, steroids, cardiac glycosides, tannins, flavonoids, and saponins was determined using methods described by Sofowora (1993) and Trease and Evans (2002).

Preparation of Culture Media

The fly feed (850 mL) was prepared in two portions, one of 600 mL and the other of 250 mL. Cornmeal (50 g) was dissolved in one part of the 250 mL portion. Yeast (10 g) was dissolved in a sufficient quantity of boiled 600 mL portion while 5 g of agar was introduced into the remaining quantity of boiled water, mixed thoroughly, and allowed to cool for 10 min. Thereafter, the initially dissolved cornmeal flour was added, mixed, and allowed for another 10 min. The dissolved yeast was also added and stirred continuously for another 10 min. The feed was then taken off the heat source and allowed to cool for 5 min. Lastly, ethanolic nipagin (0.05% w/v) was then added to the feed.

Six (6) different concentrations (4 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 20 mg/mL and 150 mg/mL) of both freeze-dried and steam-dried extracts of *S. siamea* (Tradomal®) were each prepared and incorporated into separate 10 g of the liquid diet to give concentrations of 20 mg, 30 mg, 40 mg, 50 mg and 75 mg respectively per 10 g of the liquid diet before dispensing into feed vials. The media in each vial was covered to keep out wild flies and kept overnight to cure. After 24 h, 20 flies were introduced into each of the previously prepared vials containing solidified meal.

7-Days Acute Toxicity Study

The effect of the freeze-dried and steam-dried extracts on fly mortality was evaluated by daily counting of the number of dead flies in each vial over a period of 7 days (Bagu *et al*., 2020; Ehigiator *et al*., 2021).

Eclosion Study

The effect of the two extracts on *Drosophila melanogaster* reproduction was assessed by eclosion scoring (Nguyen and Moehring, 2015). Eclosion scoring was carried out by counting the number of empty pupal cases from which the flies must have emerged at the end of the first reproductive cycle (i.e., about 12 days after the commencement of the experiment).

Data Analysis

Data on eclosion score was analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni *post hoc*. Analysis was conducted using GraphPad Prism software. Median lethal concentration (LC_{50}) was determined using an online LC_{50} calculator (AAT Bioquest, 2021).

RESULTS

Phytochemicals Present in Freeze-dried and Steam-dried Aqueous Extracts of *Senna siamea*

The phytochemical screening of both extracts revealed the presence of saponins, tannins, flavonoids, alkaloids, carbohydrates, and steroids. Anthraquinones and cardiac glycosides were absent in both extracts.

LC_{50} of Freeze-dried and Steam-dried Aqueous Extracts of *Senna siamea*

Figures 1 and 2 show the LC_{50} values of both steam-dried and freeze-dried extracts of Tradomal® to be 2767.83 mg/10 g and 4475.74 mg/10 g of the diet respectively in drosophila flies.

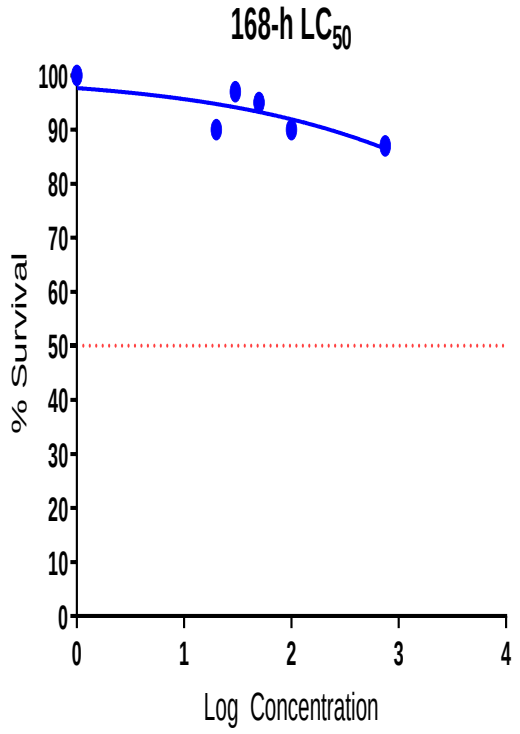

Figure 1: Determination of the median lethal concentration (LC_{50}) of the steam-dried extract of Tradomal® in *D. melanogaster*.

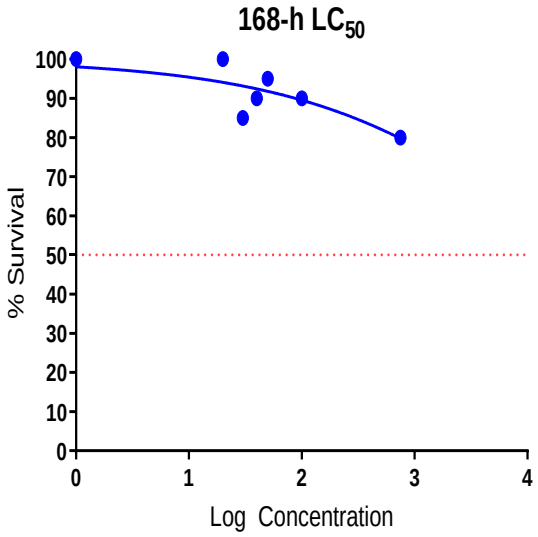

Figure 2: Determination of the median lethal concentration (LC_{50}) of freeze-dried extract of Tradomal® in *D. melanogaster*.

Effect of Freeze-dried and Steam-dried Aqueous Extracts of *Senna siamea* on Eclosion

The steam-dried aqueous extract of *S. siamea* at all concentrations caused a comparable number of flies to metamorphose into adults (Figure 3). However, the freeze-dried extract significantly ($P<0.05$) affected eclosion at all concentrations except at 20 and 50 mg/10 g concentrations (Figure 4).

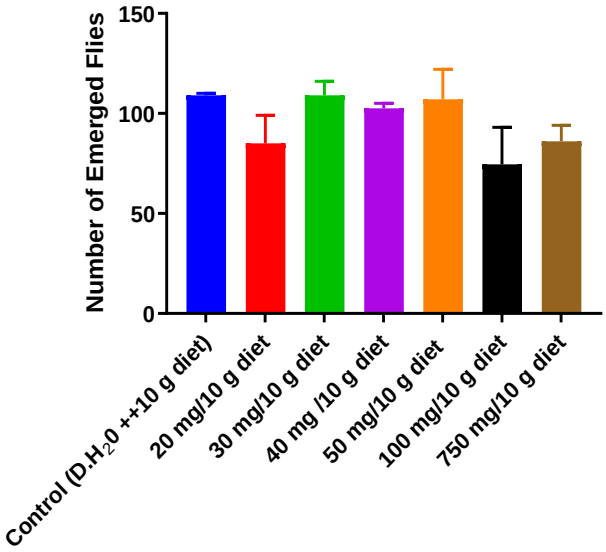

Figure 3: Effect of steam-dried extract of *S. siamea* (Tradomal®) on the emergence of *D. melanogaster flies*.

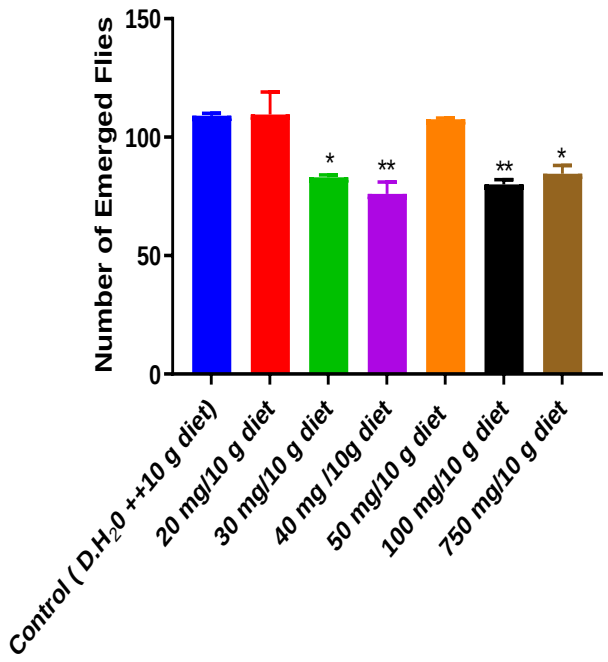

Figure 4: Effect of freeze-dried extract of Tradomal® on the emergence of *D. melanogaster* flies.

DISCUSSION

The toxicity study revealed an LC_{50} value greater than 1000 mg/10 g but less than 5, 000 mg/10 g diet for both freeze-dried and steam-dried extracts of *S. siamea* (Tradomal®). According to the Hodge and Sterner (1949) scale of toxicity, this is an indication of slight toxicity and contradicts the anecdotal claim of the absolute safety of Tradomal®.

The phytochemicals present in any plant are responsible for its therapeutic effect, pharmacological activity, and or toxicity (Mendoza and Silva, 2018). Both steam-dried and freeze-dried extracts of *S. siamea* were found to contain the same phytochemical constituents. However, the freeze-dried extract significantly inhibited fly eclosion when compared to the steam-dried extract. The yield and composition of phytochemicals in an extract are influenced by the method of concentrating the extract. A study by Abdullahi *et al.* (2012) showed how the phytochemical yield of *Orthosiphon stamineus* was affected by the drying method employed to concentrate the crude aqueous extract. The reproductive toxicity of the freeze-dried extract may be attributed to higher levels of toxic phytochemical(s) and or lower levels of protective phytochemicals. Moreover, drying methods such as oven-drying, sun-drying, and steam-drying, which employ higher temperatures are known to destroy heat-sensitive phytochemicals while methods such as freeze-drying preserve these secondary plant metabolites (Azwanida, 2015). It is therefore possible that some of the toxic metabolites that are heat-sensitive were lost during the steam-drying process making the extract less toxic. It is also possible that freeze-drying preserved the toxic phytochemicals thereby making them more toxic.

CONCLUSION

Both freeze-dried and steam-dried aqueous extracts of *S. siamea* (Tradomal®) are relatively safe in drosophila fly and the method of drying the aqueous extract can influence the toxicity of the plants. Further studies are needed to be conducted to identify and quantify the specific phytochemicals present in both extracts.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS CONTRIBUTIONS

The study was conceptualized by MOM. Experiments were performed by MAS and SOS supervised by MAM. MAS wrote the manuscript but was read by all the authors who all agreed to the contents and approved of them.

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