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Full Length Research Paper

Anti-asthma potential of dried *Draco Spilopterus* Wieg. 1834 (Philippine Flying Dragon) using mesenteric mast cell count by atopic allergy method

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Decocted *Draco spilopterus* has been utilized in the Philippines for the treatment of asthma associated with its strong folkloric beliefs however no scientific evidence was available to prove such claims. Thus, the researchers ventured into this study to determine the anti - asthma potential of dried *D. spilopterus* on male albino rats using mesenteric mast cell count by atopic allergy method. Fifteen *D. spilopterus* were used in the study. Decocotion was the extraction method employed. Seven male albino rats were used in each trial and randomly divided into different test groups which were as follows: Treatment groups (600, 800 and 1000 mg/kgbw); negative control group (water); positive control group (Prednisolone 10 mg/kgbw); untreated group; and normal group. Mesentric mast cell count by atopic allergy method was employed in this study. Results revealed that the test solutions with a dose of 600, 800 and 1000 mg/kgbw elicited a mean anti-asthmatic activity of 42.25, 66.44 and 89.32%, respectively. The median effective dose was 663.90 mg/kgbw. The anti - asthma activity was dose - related; with increasing dose, the disrupted mast cell decreases. The test solution obtained from *D. spilopterus* is a potential alternative in the management of asthma but further studies have to be conducted.

Key words: Draco spilopterus, mesenteric mast cell count.

INTRODUCTION

Asthma is a chronic disease that affects the airways of the lungs characterized as airway hyper responsiveness and airflow obstruction at the bronchial level (Blitski et al., 2013; Nimgulkar et al., 2011; Varona et al., 2014). According to the World Health Organization (WHO) estimates, 235 million people suffer from asthma. It is a public health problem in all countries regardless of level of development. Over 80% of asthma deaths occur in low and lower - middle income countries, such as the Philippines. According to the WHO data in May 2014,

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asthma deaths in the Philippines reached 2.37% of total deaths. Asthma belongs to the top 10 causes of death in the Philippines. In a study by Lai et al. (2006) the estimated direct per - patient cost of asthma, including maintenance, urgent care, and drug as the basis, in the Philippines was $212±11. The health care expenditure for the treatment for asthma in the Philippines is costly. Furthermore, drugs used in the management of asthma are associated with several drawbacks. Inhaled corticosteroids work quickly and effectively in about 95% of patients with acute asthma. While it is challenging to beat the efficacy of inhaled steroids, new drugs should better them on their side effects profiles, especially when used frequently in a long - term fashion. There is still a concern about the use of inhaled corticosteroids as patients fear long - term side effects such as osteoporosis and stunting growth in children (Barnes, 2009). Thus, this necessitates the search for new therapies for asthma, driven by the prospect of a significant need for anti - asthma medications globally.

We ventured into this study to provide another management regimen for asthma which is native and rich in the Philippine forests to help especially those who are located in remote areas with difficult access to hospitals and medical care facilities. Influenced by folkloric belief originating from the ancient Chinese, the *D. spilopterus*, also known as the Philippine Flying Dragon, could effectively cure asthma. With this belief, some Filipinos have adapted this kind of regimen. The belief has been and is still practiced in the Philippines. However, no scientific evidence was available to prove such claims. Thus, this study was conducted, for the first time, in order to determine the number disrupted mesenteric mast cells of the rats (Figure 3), in relation to the treatment groups; determine which among the doses would produce the least disruption on the mesenteric mast cells; determine the percent activity of the test solutions; lastly, determine the median effective dose of the test solutions (Figure 2).

MATERIALS AND METHODS

Collection and preparation of the animal sample

The dried *D. spilopterus* were purchased in a store located along Osmeña Boulevard, Cebu City, Philippines. They were authenticated by the Department of Biology, University of San Carlos. They were washed to remove adhering materials and cut into small pieces.

Extraction of the animal sample

The extraction method used was decoction. Fifteen cut dried *D. spilopterus* were placed in a pot. Water was added and was brought to a boil for 15 to 20 min. Once it boiled, the pot was covered and removed from the heat. The stove was set to lowest heat and the liquid was allowed to simmer for about 45 min. Once the end of the simmer time was reached, the pot was removed from heat and was set aside for about two hours to cool down. The boiled flying lizards were strained out of the liquid and the remaining extract was squeezed out. The filtrate was evaporated to dryness using an evaporating dish using a water bath.

Preparation of the stock solution

About 9.6 g of dried powder extract from the boiled *D. spilopterus* was weighed and dissolved in sufficient amount of water to obtain a 100% w/v stock solution. From the stock solution, three different arbitrary doses of 600, 800 and 1000 mg/kgBW of rat, were administered to the treatment groups.

Preparation of the positive and negative controls

Prednisolone (20 mg/5 ml) syrup was used for the dose adjustments of the individual rats. Only one dose served as the positive control (10 mg/kg). The National Asthma Education and Prevention Program Expert Panel recommend 40 to 180 mg/day P.O. dosage of prednisolone. The dose was calculated by the average therapeutic dose of humans to rat on the basis of BSA (conversion factor - 0.018 for rats) by referring to the table of Paget and Barnes (1964). The negative control was plainly water with a dose of 0.2 ml/20 g.

Test animals

All test animals were housed in the University of San Carlos animal house and the research protocol was conducted under the supervision of a certified and trained animal technician ensuring proper animal handling was observed throughout the duration of the study. Male albino rats weighing about 100 to 200 g were selected. Prior to experimentation, the rats were divided into seven groups (Table 1) each containing three rats and then acclimatized for three days with a diet of standard rat pellets.

Mesenteric mast cell count by atopic allergy method

The procedure adopted for mesenteric mast cell count is by atopic allergy method in accordance with the study of Reddy et al. (2010) and Balaji et al. (2014).

Sensitization of the test animals

After three days of acclimatization, the rats were sensitized only on the first day of experimentation by injecting 0.5 ml of horse serum and 0.5 ml of triple antigen, subcutaneously, specifically near the abdomen. The horse serum was for the induction the allergic reaction, which was supported by the administration of the antigen.

Administration of doses

Group 1 was used as negative control without any drug treatment and Group 2 was used as positive control and was administered with prednisolone, 10 mg/kg body weight, orally. Groups 3, 4 and 5 were treated with 600 mg/kg body weight, 800 mg/kg body weight and 1000 mg/kg body weight of the test solution prepared from *D. spilopterus* extract. Group 6 was used as the untreated group and therefore received no treatment. Group 7 was used as the normal group and was not sensitized by the allergen and no treatment was received. Complete allergic reaction of the horse serum occurs after 5 to 24 h after administration. After sensitization, the rats were treated with prednisolone and test solutions of different doses from the 5th to 12th day.

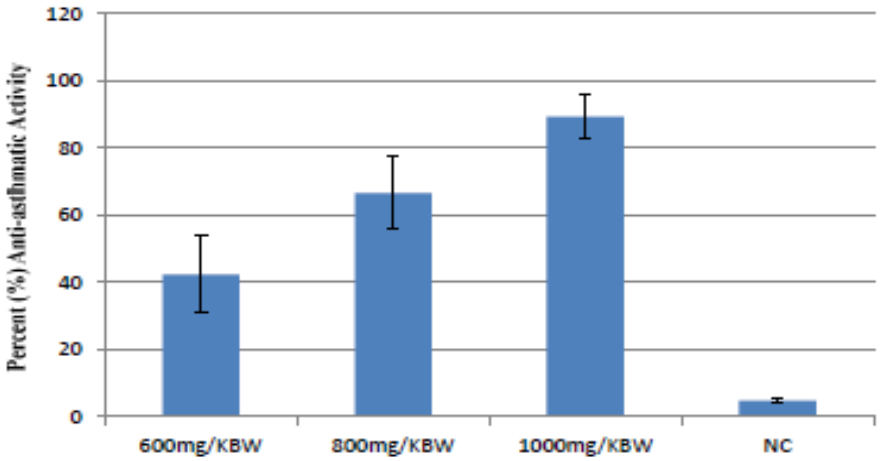

Figure 1. Percent anti-athma activity.

Staining of the mesenteric mast cells

On the 13th day, all rats from each group were sacrificed for microscopic examination on the mast cells. The mesenteries of the sacrificed animals along with pieces of intestine were suspended in Krebs-Ringer solution in order to obtain free, membrane-bound granules for rupturing cells. The mesentery pieces were exposed with 5% horse serum for 10 min. Pieces of mesentery were stained superficially with toluidine blue for mast cells to stain metachromatically. The cells stained a different color from the dye solution and the rest of the tissue. Mast cells stained red-purple (metachromatic staining) and the background stained blue (orthochromatic staining). Metachromasia, tissue elements staining a different color from the dye solution, is due to the pH, dye concentration and temperature of the basic dye. Blue or violet dyes will show a red color shift, and red dyes will show a yellow color shift with metachromatic tissue elements. The tissue was immersed in 0.1% toluidine blue (in 4% aqueous saline) for 10 min. The tissue was cleared in xylene for 5 to 10 min. It was rinsed with acetone thrice and was placed on a microscopic slide, stretched with the help of a needle. The intestinal pieces were cut and removed.

Counting of disrupted mast cells

The tissue was examined under the microscope in 100X magnification. Three slides per animal were used. Disrupted mast cells were stained red-purple and the normal mast cells were stained blue. Each slide was divided in three grids and the disrupted mast cells in four randomly selected fields for each grid were counted. With the numbers of disrupted mast cells, the percent anti-asthmatic activity was calculated using the formula shown below. Anti-asthmatic activity shown by the negative control is considered negligible:

$$Percent\ Activity\ (positive\ control) = \frac{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

$$Percent\ Activity\ (treatment\ groups) = \frac{Untreated_{disrupted\ mast\ cell} - Test\ solution_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

$$Percent\ Activity\ (negative\ control) = \frac{Untreated_{disrupted\ mast\ cell} - Negative\ control_{disrupted\ mast\ cell}}{Untreated_{disrupted\ mast\ cell} - Positive\ control_{disrupted\ mast\ cell}} * 100$$

Median effective dose (ED_{50})

A linear regression analysis was used to obtain a linear equation through Microsoft Excel 2007 that will be then be used to calculate ED_{50}.

Statistical analysis

Statistical analysis was performed using Microsoft Excel software and StatPlus 2009 program. To confirm the variability of the data and validity of results, the number of disrupted mast cells was compared using one-way analysis of variance (ANOVA). Differences between the untreated and the test solutions were considered statistically significant at standard *P* value ≤ 0.01.

RESULTS AND DISCUSSION

The test solution of *D. spilopterus*, based on the results, indicates an anti-asthma property. This was exhibited by the number of disrupted mast cells obtained and counted from the intestinal mesentery of the test animals. The anti-asthma activity exhibited by the *D. spilopterus* was dose-related; with increasing dose, the disrupted mast cell decreases.

The median effective dose (ED50) is 663.90 mg/KBW. Theoretically, the dose in which 50% anti-asthmatic activity will be exhibited by the test solutions is 663.90 mg/KBW. Linearity was verified by analysis of different concentrations. As a result, regression analysis showed good correlations with R^2 = 0.9997 in the doses 600, 800 and 1000 mg/KBW. This means that there is a direct relationship between the dose and the percent anti-asthmatic activity (Figure 1).

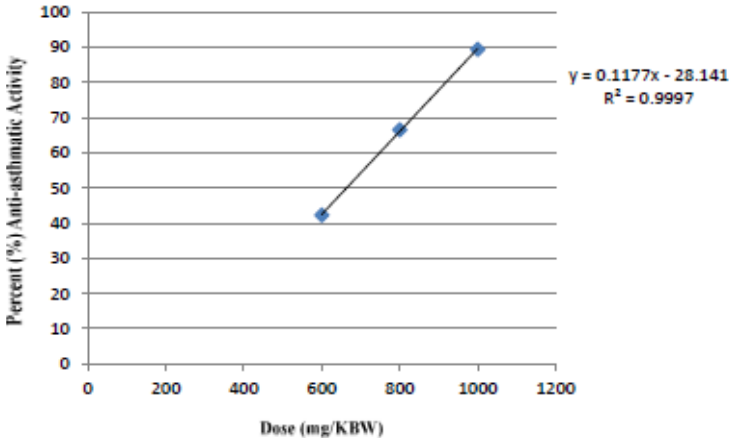

Figure 2. Median effective dose.

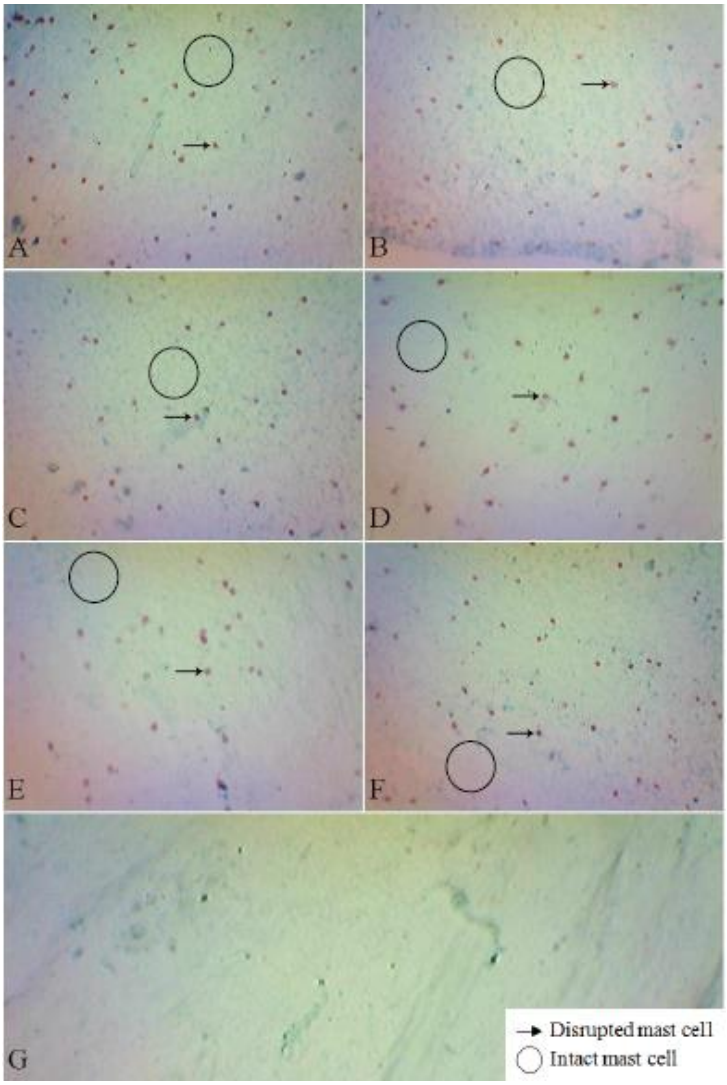

Figure 3. Comparison of disrupted mast cells among the positive control, negative control, test solutions and untreated; A: Negative Control; B: Positive Control; C: 600 mg/KBW; D: 800 mg/KBW; E: 1000 mg/KBW; F: Untreated; G: Normal.

Table 1. Treatment groups.

Treatment groups	Treatment
1	Negative control (Water - 0.2/20 g)
2	Positive control (Prednisolone - 10 mg/KBW)
3	Test solution (600 mg/KBW)
4	Test solution (800 mg/KBW)
5	Test solution (1000 mg/KBW)
6	Untreated (Sensitized without treatment)
7	Normal (No sensitization and treatment)

Table 2. One-way ANOVA for percent anti-asthma activity.

Source of variation	d.f.	SS	MS	F	p-value
Between Groups	3	11,760.8268	3,920.2756	53.4157	0.0000123
Within Groups	8	587.1346	73.3918		
Total	11	12,347.9614			

A probability (*p*) value of 0.0000123 was obtained. A *p*-value of < 0.01 indicates that a statistical significant difference exists between the different doses in relation to their anti-asthmatic activity. This indicates that the observed difference in outcomes is due to the observed effect. The treatment groups have a dose dependent relationship. As the dose increases, the percent anti-asthmatic activity also increases (Table 2).

The probable mechanism of *Draco spilopterus*'s anti-asthma activity is that it offers significant protection against degranulation by stabilizing the mast cells, which is responsible for the decreasing airway inflammation by preventing release of various inflammatory mediators. This may be due to the presence of alkaloids.

Chemical analysis on the secondary metabolites *D. spilopterus* has never been conducted however, based on literature, chemical tests was performed on another lizard species, *Tropidurus semitaeniatus*, utilized in traditional medicine in Northeast Brazil which revealed that it contains alkaloids (Santos et al., 2012). A study also revealed that an alkaloid produced statistically significant protection against Tween 80 - induced degranulation, as also to sensitized mast cells challenged with an antigen (horse serum) (Ghosal et al., 1986).

Based on the results, the proponents conclude that the extract of dried *D. spilopterus* in doses of 600, 800 and 1000 mg/KBW elicited an anti-asthmatic activity on male albino rats. The positive control group administered with 20 mg/5 ml prednisolone produced an average of 170 disrupted mast cells. The negative control group administered with plainly water produced an average of 343 disrupted mast cells. The treatment groups with doses 600, 800 and 1000 mg/KBW produced an average of 275, 232 and 190 disrupted mast cells, respectively. Based on the number of disrupted mast cells, the dose of 1000 mg/KBW showed the most significant relationship with the least number of disrupted mast cells. The average calculated percent anti-asthmatic activity of the test solutions were 42.25% for 600 mg/KBW, 66.44% for 800 mg/KBW, 89.32% for 1000 mg/KBW. Using the obtained percent activity, the median effective dose (ED50) is 663.90 mg/KBW.

These findings provide a preliminary data on the anti-asthma effect of decocted *D. spilopterus*. Further investigations on the isolation of specific compounds and toxicity studies may warrant the development of the animal extract into a drug product.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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