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The potential role of Red Tilapia (*Oreochromis niloticus*) scales: allergic reaction test in mice

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ABSTRACT

Red tilapia (*Oreochromis niloticus*) is one of aquaculture's most adaptive species. It is abundantly found in the wild and known to be cultured in several parts of the world. It is mainly a freshwater fish, inhabiting shallow streams, ponds, rivers and lakes but less commonly found living in brackish water. The concept of 'waste to wealth' has been applied widely as an alternative to waste reduction and environmental conservation. Based on these perspectives, the present study was conducted to assess possible medicinal and pharmaceutical values of the tilapia. The mice model of delayed-contact hypersensitivity test, the mice ear swelling test (MEST), was chosen for this study due to its ability to predict contact sensitization with less cost and time consumed. Four female BALB/c mice were tested in each group which included controls. Three different doses of scales powder at 500, 1000, and 2000 mg/kg in an acetone and olive oil (AOO) solution were formulated and applied to shaved dorsal trunk of the mice at induction phase, and to both sides of each ear of the mice at elicitation phase. From the observations made throughout the study period, neither erythema nor oedema was formed on the skin of mice treated with scales powder in AOO solution. Mice ear thickness increase showed percentage ear swelling of no more than 20%. Thus, the finding of this study showed that the scales of red tilapia fish did not induce allergic sensitization and could have an application in medicinal and pharmaceutical industries.

INTRODUCTION

Tilapia is mainly a freshwater fish and the red tilapia (*Oreochromis niloticus)* is native to Central and North Africa including the Middle East (Boyd 2004). It is a tropical freshwater and estuarine species. In Malaysia, red tilapia contributes approximately 90% of the total tilapia production. Tilapia has been used as biological controls for certain aquatic plant problems. In Kenya, because the tilapia consumes mosquito larvae, it was introduced to control mosquitoes which were causing malaria, consequently reducing the numbers of mosquitoes (Petr, 2000). Fish scales are one of the major wastes from fish processing that can be used widely to replace a related source or even being utilized for industry, medicinal, and pharmaceutical purposes. Wastes in the form of fish scales are being produced abundantly every day in fish markets all around the world. Guerard *et. al.,* (2001) reported that, canned fish processing creates solid wastes composed of muscles, fish viscera, gills, flesh dark/dark muscle, head, bone, and skin, which can be as high as 70% of the original material. While Kittiphattanabawon *et. al.,* (2005) reported that more than 30% of fish processing wastes consist of skin, scale and bone wastes. According to Food and Agriculture Organization (FAO, 2012), world fisheries production in 2008 touched 146 million tonnes and a recorded value of USD 103 billion. Due to the increasing health consciousness of the consumers, seafood was increasingly ingested during the past decade. According to the Malaysian Department of Fisheries, in Malaysia alone, the total fish production in 2010 was approximately 1.77 million tons at a value of RM 6.8 million. As the total of world fisheries is expected to increase in the following years, it is an effort worth taken to convert the large quantities of fish waste into beneficial products. Red tilapia fish is common and very popular among local people in Malaysia. Thus, many of its wastes are easily found in the fish markets in this country. These days, people have started utilizing by-products of almost every plant and animal, which includes that

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from fisheries as an alternative to waste reduction and environmental conservation by applying the concept of 'waste to wealth'. For example, gelatine is produced from marine fishes and chitin and chitosan from shell waste of shellfishes for biomedical purposes. Herpandi *et. al.*, (2011) claimed that the production of gelatine from fish wastes is an area that has gained much attention, especially beneficial materials that can be obtained from fish skin due to its properties and qualities.

Prior to the utilization of fish scales commercially especially in pharmaceutical products, sensitization studies are essential, as it will serve as a platform in demonstrating the potential of a material in eliciting a delayed-type hypersensitivity (DTH) immunological response through contact with the skin. These allergic or hypersensitivity reactions involve immunological mechanisms and studies to determine skin sensitization and tests may be performed using either specific chemicals from the test material, the test material itself, or most often, extracts of the test material. Up to now, there is limited information related to skin reactions caused by fish scales. Chiou and Tschen (1993) reported that fish scales from the blue gill (*Lepomis machrochirus*) are able to induce an irritant dermatitis through initial adhesion by mucopolysaccharide secretions. The scales physically adhered to the skin and formed a raised fold of skin within minutes after placement. A sub-acute irritant dermatitis was observed that later evolved into a chronic dermatitis with hyperkeratosis. However, inverted fish scales (the anatomically reversed surface) and fish epidermis did not produce such lesions on the mice. The aim of the present work was to study whether the scales of the red tilapia induce allergic sensitization on the skin when in direct contact with the body of mouse.

MATERIALS AND METHODS

Materials

Chemicals

Acetone (C_3H_6O) ChemAR® Systerm®, Diethyl ether ($(C_2H_5)_2O$) mbG® Chemicals, Olive oil Bertolli, Italy, picric acid ($C_6H_3N_3O_7$) Xylene (C_6H_4 $(CH_3)_2$) MERCK Germany, and other chemicals used in this experiment were of analytical grade.

Experimental Animals

The experiment was performed on adult female BALB/c mice weighing 15-25 g. The animals were used for experiments after two weeks of acclimatization. Animals were kept under standard laboratory conditions at a temperature of 22 ± 3°C, relative humidity of 30 – 70%, 12/12 light-dark cycle and had free access to tap water and standard pelleted food. The study was performed according to the Guidelines for Animal Study and was approved by the Faculty of Allied Health Sciences Research Committee of the institution.

Experimental Design

Twenty four adult female BALB/c mice were divided randomly into six groups, each group contained four mice. Groups were 1. Positive controls - 0.03 ml xylene; 2. Controls - Acetone and olive oil (AOO); 3. Negative controls - Untreated; 4. Red tilapia (*Oreochromis niloticus*) scales powder in AOO solution at 500 mg/kg; 5. Red tilapia (*Oreochromis niloticus*) scales powder in AOO solution at 1000 mg/kg; 6. Red tilapia (*Oreochromis niloticus*) scales powder in AOO solution at 2000 mg/kg.

METHODS

Sample Collection

Fresh red tilapia (*Oreochromis niloticus*) fish that weighed an average of 4.5 kg were bought from the local wet market in Kuantan, Malaysia. Fish samples were cleaned with running tap water and transported immediately to the laboratory on ice.

Sample Preparation

Scales were removed manually by using a fish scaler and the collected scales were cleaned and wet weight recorded. Scales were then placed in an oven of ±40 °C and left for 48 hours to dry. The yield of dried scales was weighed to obtain the dry weight and the scales were kept in the dryer for further processing.

Sample Size Reduction

The size reduction step was carried out in order to reduce the size of scales and turn it into fine powder form. Scales were ground using a grinder and sifted through a 63 μm sieve, so that only the finest particles of the scales were collected, and the weight was recorded. Fine scales powder was kept in a Schott bottle in a ±4 °C cold room.

Dose Formulation

The doses of red tilapia scales were expressed as weight of test substance per unit weight of test animal (mg/kg). Tables 1, 2 and 3 show the formulated doses that were administered to the mice dorsal trunk at the induction phase and to both ears at the elicitation phase.

Table 1: Dose formulated for Group - 4 (500 mg/Kg) mice.

Mice No	Mice Weight (gm)	Dose mg/kg
1	21.3	10.65
2	21.0	10.50
3	23.6	11.80
4	21.0	10.50

Table 2: Dose formulated for Group - 5 (1000 mg/Kg) mice.

Mice No	Mice Weight (gm)	Dose mg/kg
1	20.0	20.00
2	18.4	18.40
3	19.0	19.00
4	23.4	23.40

Table 3: Dose formulated for Group - 6 (2000 mg/Kg) mice.

Mice No	Mice Weight (gm)	Dose mg/kg
1	19.0	38.00
2	19.5	39.00
3	20.9	41.80
4	20.0	40.00

Vehicle Preparation

Acetone and olive oil (AOO) solution was used as the vehicle in this study. It was prepared by mixing four parts of acetone with one part of olive oil. The solution was mixed thoroughly using a magnetic stirrer and kept in a Schott bottle. As the test substance in this study was in a solid form, it was moistened sufficiently with a suitable vehicle to ensure good contact and absorption through the skin.

Sensitization Assay

Mouse ear swelling test (MEST)

The sensitization assay was in accordance with the hypersensitivity test methods by the National Toxicology Program (Gad *et. al.*, 1986). Approximately 24 hours before the test, fur was removed from the dorsal trunk region of the mice and care was taken to avoid abrading the skin

Test substances were administered on the shaven part of the mice for three consecutive days, which is called the induction phase. The next four days were the rest phase, and on the eighth day, an average of three readings (for each ear of each mouse) of pre-treatment ear measurement was recorded. In elicitation phase, the same doses and amount of the scales powder, xylene and AOO solution as in the induction phase were administered to both sides of right and left ears of each mice.

Observation and Collection of the Data

Observations were made on the first hour after each administration of the test substances as well as day 2 until day 10 for any erythema or oedema formation on the dorsal trunk of the mice and on day 9 and 10 for any erythema or oedema formation on the ears.

Post-treatment mice ear measurements were made 24 and 48 hours after application of the test substances in the same manner as the pre-treatment, by using the Mitutoyo micrometer. Mice were anaesthetized with diethyl ether in order for the ear measurements to be taken accurately.

The percentage (%) of ear swelling was calculated as follows:
[Mean thickness of both ears (24 or 48 hours post-treatment) /
Mean thickness of both ears measured pre-treatment × 100] – 100

The % ear swelling for the test substance was compared to the % ear swelling for the positive control, negative control as well as the vehicle for significance and dose response.

STATISTICAL ANALYSIS

A statistical analysis was done to determine the significance of the study results. The statistical test used in this study was the independent-samples t-test to analyse and compare the data between the groups of mice treated with 500, 1000, and 2000 mg/kg of red tilapia (*O. niloticus*) scales powder with xylene (control). Ear thickness increase is expressed as mean ± standard error mean (S.E.M.) % ear swelling with $p < 0.05$ set as the limit of significant difference.

RESULTS

MEST for Delayed-Contact Hypersensitivity

Mice ear thickness increase is expressed as % ear swelling for all groups of mice. Mean for ear thickness changes in mice of each treatment group in MEST for the three different doses of red tilapia (*O. niloticus*) scales powder in AOO solution, xylene and the untreated group are presented in Fig. 1 supported with actual values recorded in Table 4. All of the mice in each treatment and control groups registered ear thickness increase but none greater than 20%.

Among the treated and untreated groups, mice treated with 0.03 ml xylene (positive control) showed the highest increment in ear thickness by 4.81% at 24 hours post-treatment, with all of the four mice's ears in the group having had slight erythema within the first hour after xylene was administered (Fig. 2). The MEST using 500, 1000 and 2000 mg/kg of scales powder in AOO solution showed ear thickness changes of 2.83 %, 3.2 6%, and 4.59 % at 24 hours post-treatment and a reduction to 2.21 %, 3.04 %, and 4.08 % at 48 hours post-treatment respectively. None of the mice's ears developed erythema.

Mice treated with AOO solution (vehicle control) showed ear thickness increase by 2.01% at 24 hours post-treatment but displayed no erythema formation. Ear thickness increase was also recorded among the mice in the untreated (negative control) group, with a percentage increase of 1.67% at 24 hours post-treatment with no erythematous ears. No oedema formation was reported throughout the elicitation phase.

Table 4: Sensitization test of red tilapia (*O. niloticus*) scales powder (500, 1000, and 2000 mg/kg) in AOO solution, xylene (positive control) and untreated (negative control) on mice ear thickness increase at 24 and 48 hours post-treatment.

Group	Ear thickness increase (% swelling) ± S.E.M.	
	24 hours	48 hours
500 mg/kg	2.83 ± 0.8713 *	2.21 ± 0.3639 *
1000 mg/kg	3.26 ± 0.6544 *	3.04 ± 0.8945 *
2000 mg/kg	4.59 ± 0.7792 *	4.08 ± 1.8792 *
Xylene	4.81 ± 0.8495	4.23 ± 1.1096
Untreated	1.67 ± 0.2985	1.09 ± 0.4187

* $P > .05$ (two-tailed).

An independent-sample t-test was conducted to compare each of the three different doses of red tilapia (*O. niloticus)* scales powder-500, 1000, and 2000 mg/kg-with xylene (control) as seen in Figure 1. There were no statistically significant differences ($p > 0.05$) in % ear swelling following application of all three different doses of scales powder in comparison to xylene.

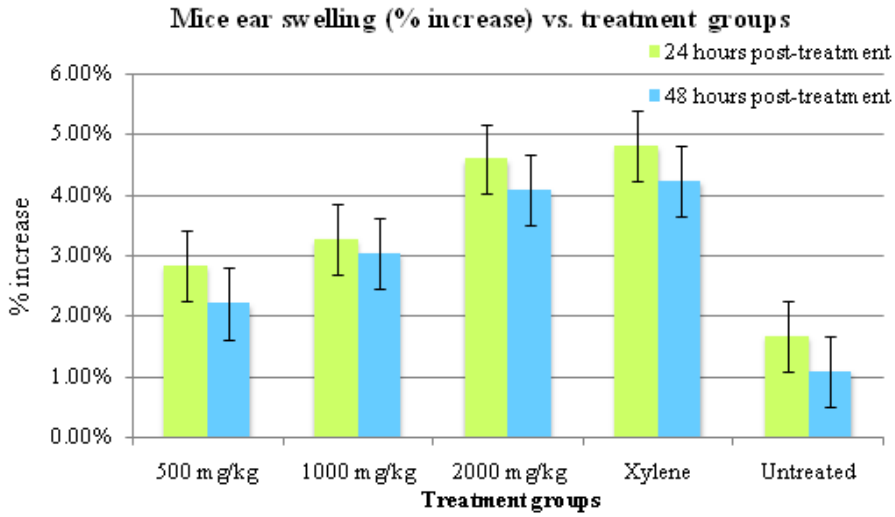

Fig. 1: Mean mice ear swelling (% increase) vs. treatment groups at 24 and 48 hours post-treatment. Error bars represent the standard error mean.

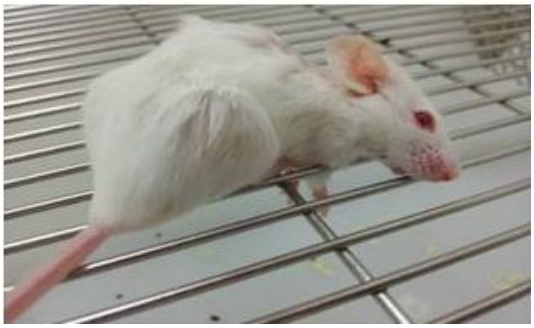

Fig. 2: Slight erythema on mouse ears within the first hour of treatment with 0.03 ml xylene.

Fig. 3: Fig. 3: No erythema formed on mouse ears tested with 2000 mg/kg of scales powder.

Fig. 4: Erythema formed on a mouse dorsal trunk within the first hour of treatment with 0.03 ml xylene.

Fig. 5: Representation of no signs of irritation observed on dorsal trunks of mice in the untreated group and all groups treated with scales powder.

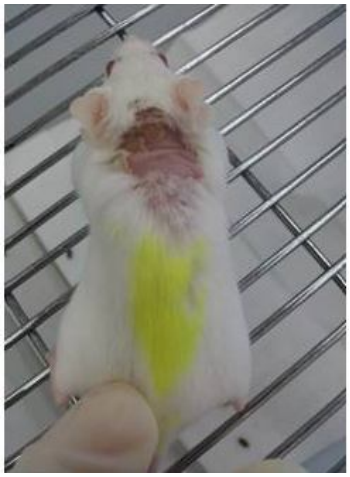

Fig. 6 Lesion on dorsal trunk of mouse treated with 0.03 ml xylene seen at the third induction day.

Induction Phase Reactions

During the induction phase, erythema was observed on the dorsal trunk of the mice tested with xylene within the first hour after administration as seen in Figure 4. No signs of irritation were seen on the dorsal trunks of the mice in the untreated and other treatment groups (Figure 5). Lesions with dry skin and brown-yellowish crusts on dorsal trunk of mouse treated with 0.03 ml of xylene were noted from the third induction day onwards (Figure 6). However, healing can be seen during the rest phase and by the completion of the study at day 10**.** Oedema formation was absent throughout the induction phase as well as the rest phase.

DISCUSSION

This study was conducted using the powder of red tilapia (*O. niloticus*) scales which was easily prepared in the laboratory. It is imperative to assess whether the scales of this kind of fish could induce any skin reaction to assess its potential for conversion into a beneficial product for the human use. Results from groups of mice treated with scales powder were compared to those of xylene (positive control), AOO solution (vehicle control), as well as the untreated group (negative control). It was found that doses of the red tilapia scales powder at 500, 1000, and 2000 mg/kg did not induce any skin reaction, neither erythema nor oedema throughout the ten days of the study period. There were increases in ear thickness as the doses were increased, from 2.83% to 4.59% at 24 hours post-treatment complying with the principle of dose-response relationship in which larger doses will produce larger effects. However, none of the increase in ear thickness was more than 20% thus considered as a negative response, based on the standard fixed by Shelton *et al.* (2006) in their study.

Xylene was chosen as the positive control for comparative purpose and for its ability to induce oedema as it is commonly used in anti-inflammatory studies. Observations and results collected from the group of mice treated with xylene showed some irritant reactions on all of the mice. During the induction phase, erythema can be seen on the dorsal trunk of the mice within the first hour after xylene application and later in the elicitation phase, the entire group of mice displayed erythematous ears within the first hour of xylene administration. The irritant reactions by xylene caused lesions with brown yellowish crusts on the mice dorsal trunk that were visible at day 3 onwards. Ear thickness increase in the xylene treated group which was recorded at 24 and 48 hours post-treatment was no greater than 20%. This was taken as a negative response to skin sensitization. The responses to xylene occurred at an early time point which implied that the reaction is more likely to be skin irritation rather than sensitization. This is further supported by the independent-samples t-test done in which the effects resulting from the application of red tilapia scales powder at different doses showed no statistically significant differences ($p > 0.05$) when compared to the responses to xylene. The most appropriate positive control for sensitization studies would be dinitrochlorobenzene (DNCB) or dinitrofluorobenzene (DNFB) for its property as a skin sensitizer and inducers of a type IV hypersensitivity reaction. Gad *et al.* (1986) and Maurer *et al.* (1994) reported that test animal sex and choice of vehicle did not have a noticeably influence on the outcome of the test. Therefore, the use of AOO solution as a vehicle in this study in order to ensure that the test substance was in contact with the skin and absorbed, is deemed not to have much an effect on the results.

Increase in ear thickness was also recorded among the mice in the untreated group. There are a number of possible reasons that might lead to this consequence. It may be due to the measurement error of mice ear using the Mitutoyo micrometer. The Mitutoyo micrometer is a precise type of measuring equipment and it is used widely in MESTs but any slight movement of the mouse might affect the readings taken. In this study, mouse was anaesthetized with diethyl ether causing it to be unconscious for a few minutes only. Other than that, mice ear blood capillary might had expanded due to stress of any mishandling thus leading to the increase in thickness while measurements were taken.

This study was conducted to demonstrate whether the red tilapia *(O. niloticus*) scales can pose a threat and become a health hazard agent if formulated for human consumption or use. On the whole, scales of the red tilapia (*O. niloticus*) were found not to be a potent skin sensitizer as no reactions on the dermal tissue were recorded when the scales were in contact with the body. However, the negative results in mouse tests have yet to be confirmed using a guinea pig method (Maurer *et al.*, 1994). It is possible that these scales may be skin irritants as there were increases in mice ear thickness recorded for all of the mice but no skin reactions were observed on dorsal trunk and ears of the mice. In another closely related work, the scales of the blue gill (*Lepomis machrochirus*), can physically adhere due to its natural tendency to curl into the skin due to the presence of an adhesive matter. A raised fold of skin was seen to later form within minutes of placement of the blue gill scales and punctuate erythematous lesions were observed on mice at day 2 (Chiou and Tschen, 1993). All biopsy specimens in the study suggested signs of acute irritant dermatitis. However, inverted blue gill scales' biopsy specimens displayed no evidence of proliferative response or inflammation. It is noted that the denuded Swiss Webster mice used in that particular study did not have prior sensitization. Similar research can be done in the future to explore the hazards and benefits of the scales of red tilapia as well as that of other fishes.

CONCLUSION

Using a mouse model of delayed-contact hypersensitivity that is MEST, we have demonstrated that scales of the red tilapia (*O. niloticus*) fish did not induce allergic sensitization to the skin when in direct contact with the body. It was found that the scales are not a potent skin sensitizer when tested in three different doses of 500, 1000, and 2000 mg/kg. Although it may be a skin irritant as there were increases in mice ears thickness, no marked skin reactions were observed from the effects of the scales throughout

the study. The red tilapia (*O. niloticus*) scale is thus seen to have a potential as a pharmaceutical or other beneficial product providing us an alternative to waste reduction and as part of the effort of conserving the environment.

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