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Research Article

Anaesthetic Effects of Piper Betle Extracts on Nile Tilapia, *Oreochromis niloticus*

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Abstract

Study on *Piper betle* extracts as an alternative herbal anaesthetic in Nile tilapia, *Oreochromis niloticus* (1.44±0.20 g in BW and 4.39±1.97 cm in TL). Acute toxicity of *Piper betle* extracts with LC_{50} valve was 40.25 ml/L for 24 hr at 95% confidence intervals from 36.49-44.29 ml/L. *Piper betle* extracts concentrations were tested at 0, 5, 10, 20, 30 and 40 ml/L. The result showed that the concentration of 30 ml/L was suitable as anaesthtic because tested fish began to show stage 1 (sedation) within 5 min after exposure and fish began to died in 6 hr exposure. The recovery tested fish after exposure in 20, 30 and 40 ml/L were average 4.33±1.52 min.

Keywords: Anaesthetic, *Piper betle*, *Oreochromis niloticus*

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บทคัดย่อ

การศึกษาประสิทธิภาพของสารสกัดพลูเป็นยาสลบในปลานิล (*Oreochromis niloticus*) น้ำหนักเฉลี่ย 1.44±0.20 กรัม ความยาวเฉลี่ย 4.39±0.19 เซนติเมตร มีค่าความเป็นพิษเฉียบพลัน ในระยะเวลา 24 ชั่วโมง (24-hr LC_{50}) มีค่าเท่ากับ 40.25 มิลลิลิตรต่อลิตร (อยู่ในช่วง 36.49 – 44.29 มิลลิลิตรต่อลิตร ที่ความเชื่อมั่นที่ 95%) และทำการศึกษาสารสกัดพลูเป็นยาสลบที่ความเข้มข้น 0, 5, 10, 20, 30 และ 40 มิลลิลิตรต่อลิตร พบว่าความเข้มข้น 30 มิลลิลิตรต่อลิตรเหมาะสมที่สำหรับการใช้เป็นยาสลบเพราะสามารถทำให้ปลาในการทดลองเข้าสู่ระยะ 1 คือ นิ่งซึมไม่เคลื่อนไหวสูญเสียการทรงตัว ในเวลา 5 นาที และเริ่มพบการตายในชั่วโมงที่ 6 จึงเหมาะสมในการทำกิจกรรมด้านการเพาะเลี้ยงสัตว์น้ำในระยะเวลาไม่เกิน 6 ชั่วโมง และระยะเวลาการฟื้นสลบของปลานิลหลังจากการสลบที่ 2 ชั่วโมงในความเข้มข้นของสารสกัดพลูที่ 20, 30 และ 40 มิลลิลิตรต่อลิตร ใช้เวลาฟื้นสลบเฉลี่ย 4.33±1.52 นาที

คำสำคัญ : ยาสลบ พลู ปลานิล

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Introduction

Anaesthetic is an agent act on the central nervous system at electrical signals by inhibiting neuro electrical signals which resulted in loss of sensation (Heavner, 1981). Normally, the brain's electrical signals are a chaotic chorus at different parts of the brain communicate with each other. Several common anesthetics bind to the GABA-A receptor in the brain's neurons. They hold the gateway open and let negative charged particles flow into the cell. The negative charge builds up and acts like a log jam by keeping the neuron from transmitting electrical signals. The nervous system (reticular activating system) has lots of these gated channels, controlling pathways for movement, memory, and consciousness. This part of the brain is the most important position within the central nervous system to make an anaesthetic in animals and humans (Muir and Hubbell, 1989).

An early anaesthetic was recorded in Egypt, Asia and Middle East medical text books in the 12th century (Hirst, 2004). It was a compound which a part consisted of either opium poppy, mandrake fruit and alcohol. In 1540 AC, Paracelsus discovered diethyl ether and began experiments on animal anaesthesia. He reported that the diethyl ether could force poultry to sleep. Sir Humphrey Davy, in 1800 AC, reported the use of nitrous oxide to anaesthetize an animal. In 1824 AC, H.H. Hickman used nitrous oxide combined with carbondioxide by inhalation during a dog operation in order to reduce pain. Next, the nitrous oxide anaesthetic was began to apply in human since 1840 AC (Euliano and Gravenstein, 2004).

Application of anaesthetic in aquaculture had been established about 60 years ago for reducing stress and injury during breeding, handling, transportation and other activities which effected fish health and production (Brown, 2011). There are many anaesthetics used in aquatic animals. Their efficacy depends on species and methodology (Chandroo *et al*., 2004; Huntingford *et al*., 2006; Rose, 2002). The well-known anaesthetics are chemicals, for example, MS-222 (TMS, tricaine methane sulfonate), benzocaine, phenoxyethanol and quinaldine etc (Ross and Ross, 2008). Most of the chemical residues might accumulated in the aquatic animal products which might affected to consumers (Amani and James, 2007). In addition, the chemical anaesthetics have side effect to users, for example, irritate at direct contact on to body or headache after inhalation because of blood circulating interruption and affecting to the central nervous. Thus, Amani and James (2007) suggested herbal anaesthetic as an alternative to reduce the chemical residue in aquatic animals. For example, *Erythroxylum coca*, *Spilanthes acmella*, *Acorus calamus* and *Syzygium aromaticum* contained cocain (Ruetsch *et al*., 2001.), spilanthol (Barbasa *et al*., 2016), acorin (Panchal *et al*., 1989) and eugenol (Sarkar *et al*., 2008), respectively. These are active ingredients to CNS (Mylonas *et al*., 2005). In Thailand, the eugenol from clove oil (*S. aromaticum*) is the most popular and widely use as herbal anaesthetic in aquaculture (Roubach *et al*., 2005; Mylonas *et al*., 2005). However, the eugenol is also found in *P. betle* leaf in Piperaceae. This leaf is one of the third compositions for chewing betel nut, a traditional chewing in Asian countries as well as Thailand. Thus, the leaf is common found locally at low price. Therefore, this research would like to investigate efficacy of the *P.betle* leaf extract as a new alternative herbal anaesthetic and appropriate dosage for tilapia.

Materials and methods

1. Experimental animals

Nile tilapia (*Oreochromis niloticus*) about 30 days old were bought from fish farm in Maha Sarakham. The fish were similar age and size at 1.44±0.20 g in BW; 4.39±1.97 cm in TL. They were 100 in total and stocked in a 400 L tank (1 fish/L). The tank had an air stone. The tanks were changed 75% water every day in order to remove wastes and keep suitable water quality. The fish were fed 30% protein pellet 15% BW twice a day. They were acclimatized about 2 weeks before starting experiment.

2. Preparation of *Piper betel* extract

Piper betel leaves were collected locally in Muang district, Maha Sarakham province. They were extracted by following Leng *et al*. (2011) instruction. The leaves were washed in tap water for clean up all dirt. Next, they were air dried and then crushed into a fine piece. The 1 kg fine crushed leaf was weighed and put into an aspirator bottle. Three liters (w/v 1:3) of ethanol (95%) were added and soaked the leaf at room temperature for 72 hr with occasionally shaking. Then, the solution was filtered through No.1 Whatman filter paper. The ethanol in solvent was removed by rotary vacuum evaporator (BuchiSyncore) at 45°C. All the remaining was crude extract stored in refrigerator (4°C) until further use.

3. Experiment I : 50% Lethal concentration (LC_{50}) of *P. betle* extract on Nile tilapia

Toxicity of *P. betle* extract was investigated by static bioassay followed APHA (1998) and Dhara *et al*. (2016) in 21 containers (2L). The *P. betle* extract solutions were prepared at 7 concentrations including 0, 10, 20, 40, 60, 80 and 100 ml/L, 3 containers/concentration for each concentration. The 10 prepared fish were randomly counted and put into each container. Next, the fish behavior was observed. Dead fish were recorded and removed during 24 hr experimental period. All data were computed for 50% lethal concentration (LC_{50}) of the extract solution using Probit analysis at 95% confidence.

2. Experiment II : *P. betle* extract induced Nile tilapia anesthesia and recovery

Diluted the LC_{50} concentration from experiment I into 0, 5, 10, 20, 30 and 40 ml/L with 3 replicates/concentration. The experiment was run in static bioassay the same as experiment I. Ten fish were randomly added into each container and observed anaesthetized behaviors every 5 min for the first hour and then every 2, 4, 6, 12, 18 and 24 hr. The fish behaviors were classified into 4 stages of anaesthetized behaviors including:

Stage 0 : No effect = Normal fish

Stage 1 : Sedation = Motion & breathing reduced

Stage 2 : Anaesthesia = Total loss of equilibrium no reaction to touch stimuli

Stage3 : Death = No movement of operculum and death

Periods in either minutes or hours of the anaesthtized behaviors were recorded in each concentration along experimental duration. Recovery stage was determined after fish became stage 2 (Anaesthesia). Data were analyzed to find appropriate concentration of *P. betle* extracts to use safely for Nile tilapia.

Results and Discussion

Results

1. Acute toxicity of *P. betle* extract on Nile tilapia

In control (0 ml/L) and 10 ml/L of the *P. betle* extract found no fish die in 24 hr. While the high concentration at 60 ml/L onward, all the fish died in 1 hr. For the 40 ml/L extract found the fish reduced movement and stay tranquility in the first 5 minutes prior to die in the 4 hr onward. Probit analysis found the LC_{50} of the extract was 40.25 ml/L (range = 36.49 - 44.29 ml/L; Figure 1).

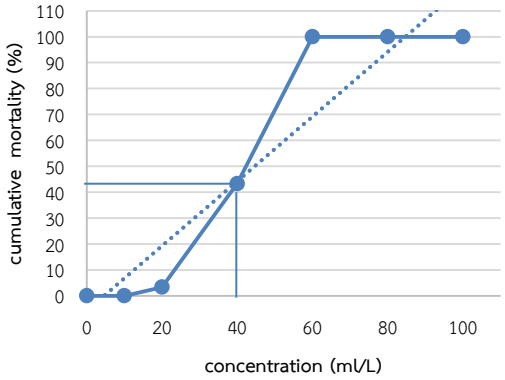

Figure 1. Cumulative mortality of Nile Tilapia after exposure to various dosages of *P. betle* extract in static bioassay for 24 hr.

2. Anesthesia induction and recovery of *P. betle* extracts exposed to Nile tilapia

In control treatment (0 ml/L) the fish showed no effect of the extract though out the 24 hr experimental period. The 5ml/L extracts, some fish began to show stage 1 (sedation) after 18 hr exposure. While at 10 ml/L extract, the fish started sedation and anaesthesia in 2 and 6 hr exposure, respectively. There were no dead fish found in this concentration. At the 20 ml/l extract the fish started stage 1 (sedation), 2 (anaesthesia) and 3 (death) in 30 min, 2 and 8 hr, respectively (Table 1). All the fish in this concentration died at 12 hr exposure. The 30 and 40 ml/L extracts, the fish began sedation in 5 min while started stage 2 anaesthesia in 20 and 15 min. The death fish were observed at 6 and 4 hr exposure, respectively. All the dead fish from 20, 30 and 40 ml/L concentrations could be recovery after immersed in a new fresh water at 4.33±0.57, 4.83±0.28 and 5.33±0.28 min, respectively.

Discussion

P. betle contained eugenol (0.16 % w/w; Hadzir and Hadzuin, 2012; Sarkar *et al*., 2008) which is part of the bioactive compound affected as an alternative fish anaesthetic. In the study, the low extract at 10 ml/L concentration affected the fish began sedation and anaesthesia in 2 and 6 hr, respectively without any death in 24 hr. The 20 ml/L extract affected the fish to calm in sedation (stage 1), anaesthesia (stage 2) and death (stage 3) in 30 min, 2 and 8 hr, respectively. The high dosages as 30 and 40 ml/L extract, the fish calmed in sedation in 5 min before went to anaesthesia in 20 and 15 min, respectively. Death fish was recorded in 6 and 4 hr of the 30 and 40 ml/L extract, respectively. Thus, the 30 ml/L extract was the effective and suitable dose for Nile tilapia. Because it was a lowest concentration that could induced anaesthesia in fish (shawn *et al*., 2004; Browm, 2011).

As Chantong *et al*. (2010) have reported that eugenol could serve as anaesthetic for carp (*Cyprinus carpio*). In addition, eugenol can reduce stress in Nile tilapia assessed by cortisol and glucose level including spontaneous superoxide anion, percent phagocytosis and antibody in Nile tilapia (Areechon *et al*., 2011). Effects of anaesthetic on fish depend on external and

internal factors of each fish species (Ross, 2001). Anaesthetic are widely used in aquaculture to reduce stress and mortality. Thus, betel extract could serve as suitable alternative fish anaesthetic.

Table 1. Behavioral events of *O. niloticus* exposed to various concentrations of *P. betel* extract in static bioassay

Concentration (ml/L)	Stages of anesthesia according to exposure periods														
	(min)							(hour)							
	5	10	15	20	30	40	50	1	2	4	6	8	12	18	24
control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0,1	1
10	0	0	0	0	0	0	0	0	1	1	1,2	2	2	2	2
20	0	0	0	0	1	1	1	1	1,2	2	2	2,3	3	3	3
30	0,1	1	1	1,2	2	2	2	2	2	2	2,3	3	3	3	3
40	0,1	1	1,2	2	2	2	2	2	2	2,3	3	3	3	3	3

* Stages of anesthesia : 0 = No effect, 1 = Sedation, 2 = Anesthesia, 3 = death

Conclusion

Efficiency of *P. betle* extract as anaesthetic in Nile tilapia was studied. The result showed that the concentration of 30 ml/L was suitable as anaesthtic because tested fish began to show stage 1 (sedation) within 5 min after exposure and fish began to died in 6 hr exposure. The recovery tested fish after exposure in 20, 30 and 40 ml/L were average 4.33±1.52 min.

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