Participation of Large Ca^{2+} Activated Potassium Channel in Antinociceptive Activity of Chalcone Derivative (3-(2, 5-dimethoxyphenyl)-1-(5-methylfuran-2-yl) prop-2-en-1-one) DMPF-1 Action in Mice Model.

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***Abstract*—**:The role of potassium channels in nociceptive activity was proposed in the past decade. Various type of potassium channel has been found to exert different action in propogation of action potential in nervous system. As DMPF-1, a chalcone derivative possesses antinociceptive properties. The mechanism of its action has been carried out to verify the pathway involved. The present study addressed the role of potassium channel in the contribution of the antinociceptive action of DMPF-1. The involvement of potassium channel was evaluated using acetic acid-induced abdominal writhing test.The animals were pretreated with charybdotoxin (large Ca^{2+} activated potassium channel blocker)(0.04mg/kg, i.p) or apamin (small Ca^{2+} activated potassium channel blocker)(0.02mg/kg, i.p.) 15 minutes before administration of DMPF-1. It was demonstrated that the challenge of DMPF-1 treated group with charybdotoxin has reversed the antinociceptive activity of this novel chalcone, which indicates the possible participation of large Ca^{2+} activated potassium channel in antinociceptive effect cause by DMPF-1 but not through small Ca^{2+} activated potassium channel.

***Keywords*-:** DMPF-1, Abdominal writhing test, Antinociceptive and Potassium channel.

I. INTRODUCTION

ACCORDING to International Association for the Study of Pain (IASP), an unpleasant sensory and emotional experience associated with actual or potential tissue damage due to chemical or thermal stimulus are the definitive of pain. Certainly, pain signaling is a defensive mechanism that alerts us to real and impending injury thus avoids further tissue damage. Even so, pain often becomes chronic and devastating as it outlives its efficacy as a warning system which bother health and functional capability of a human[1].Many analgesic drugs has been developed to overcome pain sensation but they bring to bear the toxicity and side effects such as gastric ulcer[2], liver damage, hypersensitivity and CNS effects [3]. As a consequence several alternative needs to be discover.

Chalcone is a chain of heterocyclic compound that appear abundantly in edible plants, which act as a precursor that important for synthesizing various classes of valued flavonoids. Several studies using chalcone and its derivatives have identified that they exert an important biological activity. They possess antibacterial, anticancer [4], antitumor[5], antifungal, anti-inflammatory [6] and antinociceptive properties [7] as they are capable to inhibit the synthesis of prostaglandins [8]. Because of their pharmacological properties, DMPF-1 was synthesized. From our previous preliminary study, DMPF-1 exerts an antinociceptive activity using chemical and thermal-induced nociception therefore it is necessary to figure out the mechanisms involved in this action.

II. MATERIAL AND METHOD

A. Preparation of Compound

The chalcone derivative was obtained from Institute of Bioscience, Universiti Putra Malaysia and synthesized by Claisen-Schmidt condensation reaction. The compound was purified by column chromatography using silica gel mesh size (100–200 mesh, Merck) and elution with petroleum ether and ethyl acetate.

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B.Animals

Male ICR albino mice aged 4-6 week old weighed about 20-25g being used throughout the experiments with free access to food and water ad libitum. The animals were housed in room with standard constant temperature 24 ± 1^{o}C in 12:12 hours dark light cycle.The rules of the ethical guidelines to assess the pain in conscious animals[9] was pursued after the approval by Animal Care and Use Committee, Universiti Putra Malaysia, Serdang, Selangor. Least number of animals (n=10) and stimulus (chemical) was employed as it can present the effect of the compound. The experimental animals were sacrificed once it was used throughout the study.

C. Drugs and chemical used

The subsequent drugs and chemical were used in commencing this study: glacial acetic acid (Scharlauchemie S.A.), charybdotoxin and apamin (Sigma Aldrich chemical, U.S.A). Normal saline was used in order to dissolve all the chemicals (0.9% NaCl). DMPF-1 was prepared using vehicle that made up of absolute ethanol, Tween 20 (Sigma, Aldrich) and distilled water in 5:5:90 (v/v) fractions respectively. The dose of treatment used was chosen based on our pilot experiment and the volume administered was 10 ml/kg; i.p.

D. Experimental protocol

The participation of potassium channel study was carried out using the method described previously with slide modification [10]. The groups of mice were pretreated intraperitoneally with 0.04mg/kg of charybdotoxin (large Ca^{2+} activated potassium channel blocker) and 0.02mg/kg of apamin (small Ca^{2+} activated potassium channel blocker) 15 minutes before received DMPF-1 at dose of 1mg/kg, i.p. or vehicle 10ml/kg, i.p. Acetic acid 0.6% was intraperitoneally-injected post 30 minutes of DMPF-1 treatment. The nociceptive response was characterized by the cumulative number of abdominal writhing from 5 to 30 minutes post injection of 0.6% acetic acid, i.p. The reduction in number of writhing episode indicated the antinociceptive action of the compound.

E. Statistical analysis

The result was accessible as the mean ± SEM involving 10 animals per group. The statistical significance comparison between groups was evaluated using one-way analysis of variance (ANOVA) with Dunnett's post-hoc test. All procedures were carried out using Prism 4.0 software (GraphPad Software, San Diego, CA, USA); $p<0.05$ was considered significant

III. RESULTS

The result in Fig. 1, shows that treatment of charybdotoxin at dose of 0.04mg/kg, i.p. alone does not altered the number of abdominal writhing induced by 0.6%, i.p injection of acetic acid. However it is, as the treatment group of DMPF-1 was challenge with this blocker, it reversed the antinociceptive activity of this compound.

In Fig. 2, pretreatment of the animals with apamin at dose of 0.02mg/kg, i.p. does not altered the antinociceptive effect of DMPF-1 compound in acetic acid induced abdominal writhing test induced by i.p. injection of acetic acid 0.6% concentration. There are significant different as compared to apamin alone treated group.

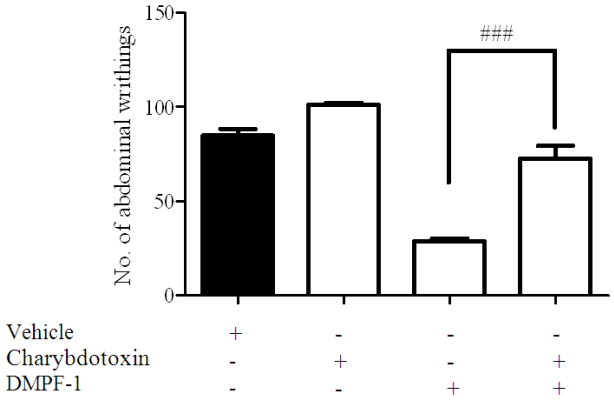

Fig.1. Effect of charybdotoxin (0.04mg/kg) on antinociception caused by DMPF-1 (1mg/kg) compound in acetic acid-induced abdominal writhing test. Each column represents mean ± S.E.M of 10 mice. ### Denotes significant difference at $p<0.001$ as compared to DMPF-1 treated group.

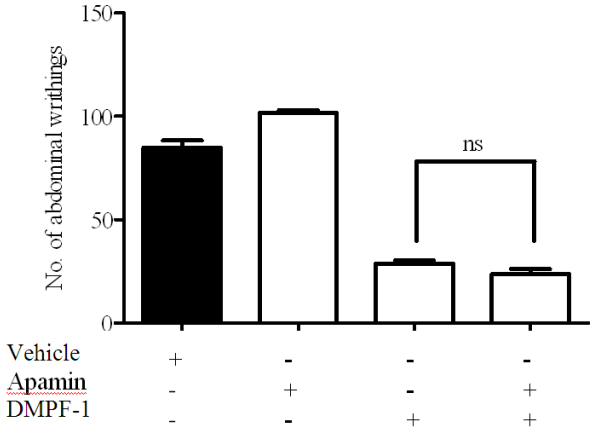

Fig. 2. Effect of apamin (0.02mg/kg) on antinociception caused by DMPF-1 (1mg/kg) compound in acetic acid-induced abdominal writhing test. Each column represents mean ± S.E.M of 10 mice. ns denotes no significant difference at $p<0.001$ as compared to DMPF-1 treated group.

IV. DISCUSSION

In this present study, the mechanism behind the action of this novel chalcone derivatives has been revealed using acetic acid induced abdominal writhing test in order to evaluate the

involvement of potassium channel in DMPF-1 induce antinociception. Acetic acid induced abdominal writhing technique was chosen in this test as it is very sensitive and useful to find the analgesic mechanism of compound. Nociception activity was triggered with the induction 0.6% acetic acid into the peritoneal cavity hint to the released of several chemicals mediator such as prostaglandin, increasing the cycloxygenase (cox) and lypoxygenase(lox) enzyme[11]. All of these mediators caused an irritation to peritoneal region thus and episode of abdominal writhing [12]

Potassium channels were found responsible in neuronal excitability[13]. They are several type of potassium channel available with different structure and function.Upon activation by the agonist, the potassium channel will open and thus permits the efflux of potassium ions from nerve cell later cause repolarization and/or hyperpolarization that decrease the induction of action potential[14]. Because of that capability, they become the therapeutic target in finding analgesic drugs. Various venom and toxin were establish to obstruct the function of potassium channels [15]. Development of selective large and small Ca^{2+} activated potassium channel blocker from venom and toxin has provided the pharmacological tool in finding the contribution of this channel in pain study.

As illustrated in fig. 1 and 2, Pretreatment of charybdotoxin and apamin alone does not modify the number of abdominal writhing in mice. The challenge of DMPF-1 with charybdotoxin; a large Ca^{2+} activated potassium channel blocker has reversed the effect of DMPF-1 which mean the possible involvement of this channel in antinociceptive action of this novel chalcone derivative.

V.CONCLUSION

In all, the antinociceptive properties of DMPF-1 was due to their potential in opening the large Ca^{2+} activated potassium channel thus reduced the excitability of the neuron and release pain sensation. These finding provide a value in DMPF-1 mechanism of action.

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