Vol. 8 No. 3 Desember 2021

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia

E-ISSN: 2580-8303
P-ISSN: 2406-9388

DITERBITKAN OLEH:
FAKULTAS FARMASI UNIVERSITAS AIRLANGGA berkolaborasi dengan
IKATAN APOTEKER INDONESIA (IAI) PENGURUS DAERAH JAWA TIMUR

Terakreditasi SINTA 2
No: B/1796/E5.2/KI.02.00/2020

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Editorial Team

56626120200
6001684

Dr. Ariyanti Suhita Dewi, S.Si., M.Sc
Editorial Board

Kementerian Kelautan dan Perikanan, Indonesia

0000-0002-9293-4091
gFILRr4AAAAJ
36159404300
6670048

Dr. Adliah Mhd. Ali
Editorial Board

Universiti Kebangsaan Malaysia, Malaysia

0000-0003-1306-8330
b4rrTOUAAAAJ
55141172000
-

Asst. Prof. Dr. Nungruthai Suphrom
Editorial Board

Naresuan University, Thailand

0000-0003-3767-8387
ZqxeBAQxUcIC
54388285100
-

Assist. Prof. Dr.rer.nat. Nuttakorn Baisaeng
Editorial Board

University of Phayao, Thailand

0000-0002-0305-5854
ylMPyhkAAAAJ
55630412100
-

Debra Dorotea, Ph.D.
Editorial Board

Karolinska Institute, Sweden

0000-0002-5302-0617
7A3VivgAAAAJ
57196217112
-

Deby Fapyane, Ph.D.
Editorial Board

Cellugy ApS, Denmark

0000-0003-1408-3030
t4FEn98AAAAJ
55711006200
-

Prof. Dr. Alfi Khatib
Editorial Board
Kulliyyah of Pharmacy, International Islamic University Malaysia, Malaysia

0000-0002-5480-0789
MAAAAJ
12140192900
-

Dr. Long Chiau Ming
Editorial Board
Universiti Brunei Darussalam, Brunei

0000-0002-6971-1383
5Gn6o5QAAAAJ
55745857500
-

Tina Tran, PharmD
Editorial Board
Temple University, USA

0000-0002-8332-8196
-
57192086006
-

Susmiandri, S.Kom
Administrative Editor
Universitas Airlangga, Indonesia

-
-
-
-

Login

Username *

Password *

Forgot your password?

Keep me logged in

Login Register

Accredited Sinta II

Instructions

- Guide for Reviewers
- Guide for Authors
- Online Submission
- Document Template
- Copyright Form

Journal Policy

- Focus and Scope
- Publication Ethics
- Article Processing Charge
- Peer Review Process
- Peer Review
- Editorial Team
- Open Access Statement
- Archiving
- Plagiarism
- Copyright
- Contact
- Old Website

Publisher

This journal has been published by the Faculty of Pharmacy, Universitas Airlangga in collaboration with IAI of East Java

Meet Our Editorial Team

Elida Zairina, S.Si., MPH., Ph.D., Apt.
Editor in Chief
Universitas Airlangga, Indonesia
-

Suciati, S.Si., MPhil., Ph.D., Apt.
Editorial Board
Universitas Airlangga, Indonesia
-

Susmiandri, S.Kom.
Assistant Editor
Universitas Airlangga, Indonesia
-

Read More

ISSN

9 772406 938003
P-ISSN

9 772580 830001
E-ISSN

Indexing

GARUDA
OCLC WorldCat
Google Scholar
DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
PKP|INDEX
sinta
Dimensions
TREND MD

Visitors

View JFIKI Stats

Keywords

Address

Fakultas Farmasi Universitas Airlangga
Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya,
(Faculty of Pharmacy Universitas Airlangga)
Jawa Timur - 60115, Indonesia

Contact Info:

Telp: 6231-5933150
Fax: 6231-5932549
Email: **jfiki@ff.unair.ac.id**

Lembaga Inovasi, Pengembangan Jurnal, Penerbitan dan Hak Kekayaan Intelektual
LIPJPHKI
Gedung AUP, Kampus C, Universitas Airlangga, Kota Surabaya, Jawa Timur, 60115

Jurnal Farmasi dan Ilmu Kefarmasian Indonesia
Pharmacy and Pharmaceutical Sciences Journal
Faculty of Pharmacy Universitas Airlangga
OPEN ACCESS
E-ISSN 2580-8303
P-ISSN 2406-9388

Vol. 8 No. 3 (2021): JURNAL FARMASI DAN ILMU KEFARMASIAN INDONESIA

Current Issue

Vol. 8 No. 3 (2021): JURNAL FARMASI DAN ILMU KEFARMASIAN INDONESIA

Published: 2021-11-30

Full Issue

PDF

Articles

The Effect of Serotonin-Norepinephrine Reuptake Inhibitor Milnacipran on Anxiety-like Behaviors in Diabetic Mice

DOI : 10.20473/jfiki.v8i32021.200-206
Tuhfatul Ulya , Chrismawan Ardianto , Mahardian Rahmadi , Dewi Wara Shinta , Junaidi Khotib
200-206
Abstract : 645
PDF : 324

PDF

Analisis Faktor-Faktor Klinik yang Mempengaruhi Kualitas Hidup Pasien Katarak di Rumah Sakit Dr. YAP, Yogyakarta

DOI : 10.20473/jfiki.v8i32021.207-216
Rizky Hidayaturahmah , Tri Murti Andayani , Susi Ari Kristina
207-216
Abstract : 812
PDF : 632

PDF

Evaluasi Penggunaan Antibiotik pada Pasien Sepsis Neonatus di Rumah Sakit X Purwakarta

DOI : 10.20473/jfiki.v8i32021.217-226
Rani Hendiyani , Wawaimuli Arozal , Hesty Utami Ramadaniati
217-226
Abstract : 530
PDF : 1247

PDF

Karakterisasi Karbamazepin Hasil Rekristalisasi Berbagai Pelarut Organik dengan Metode Slow Evaporation

DOI : 10.20473/jfiki.v8i32021.227-234
Indra Indra , Rendi Rahman , Rika Yulianti
227-234
Abstract : 1770
PDF : 1754

PDF

Uji Aktivitas Senyawa Bahan Alam terhadap Enzim Mpro pada SARS-CoV-2 Secara In Silico

DOI : 10.20473/jfiki.v8i32021.235-241
Syahrul Hidayat , Afifah Cahyohartoto , Ayu Utami Dewi , Izzah Al Mukminah , Oktavia Sabetta Sigalingging
235-241
Abstract : 875
PDF : 624

PDF

Molecular Docking of Compounds in Moringa oleifera Lam with Dipeptidyl Peptidase-4 Receptors as Antidiabetic Candidates

DOI : 10.20473/jfiki.v8i32021.242-249
Indah Permata Rendi , Gabriella Josephine Maranata , Hasna Chaerunisa , Nurulita Nugrahaeni , Siti Sarah Alfathonah
242-249
Abstract : 541
PDF : 466

PDF

Pengetahuan dan Ketepatan Apoteker dalam Pemusnahan Obat Sisa, Obat Rusak dan Obat Kadaluarsa di Apotek Malang Raya

DOI : 10.20473/jfiki.v8i32021.250-258
Hananditia Rachma Pramestutie , Ratna Kurnia Illahi , Ayuk Lawuningtyas Hariadini , Tamara Gusti Ebtavanny , Malyda Savira
250-258
Abstract : 1831
PDF : 1909

PDF

In Vitro Antimalarial Activity and Toxicity of Helianthus annuus L. Leaf Extract against Plasmodium falciparum

DOI : 10.20473/jfiki.v8i32021.259-263
Nuriha Marangoh , Suciati Suciati , Wiwied Ekasari
259-263
Abstract : 380
PDF : 204

PDF

Pengembangan Sediaan Emulgel Antioksidan dan Tabir Surya Mengandung Ekstrak Kulit Buah Cokelat (Theobroma cacao L)

DOI : 10.20473/jfiki.v8i32021.264-270
Sani Ega Priani , Rizki Anggara Permana , Mira Nurseha , Ratih Aryani

264-270
Abstract : 840
PDF : 1051

PDF

Molecular Docking of Mangostin and Sinensetin Derivatives on SUR1-Pancreatic KATP Channel Target as Antidiabetic

DOI : 10.20473/jfiki.v8i32021.271-276
Intan Kris Prasetyanti , Sukardiman Sukardiman , Suharjono Suharjono
271-276
Abstract : 395
PDF : 203

PDF

Perbandingan Metode Sintesis Senyawa 1-benzil-3-(4-etil-benzoil)urea dan 1-benzil-3-(4-klorometil-benzoil)urea sebagai Calon Obat Antikanker

DOI : 10.20473/jfiki.v8i32021.277-283
Farida Suhud , Daryono Hadi Tjahjono , Tegar Achsendo Yuniarta , Galih Satrio Putra , Melanny Ika Sulistyowaty
277-283
Abstract : 454
PDF : 335

PDF

Comparative Analysis of Actual Cost and INA CBG Rate in Diabetic Gangrene Inpatients

DOI : 10.20473/jfiki.v8i32021.284-292
Diajeng Putri Kinanti , Umi Athiyah , Yunita Nita , Muhammad Noor Diansyah
284-292
Abstract : 284
PDF : 185

PDF

Linguistic Validation of Indonesian Version of the Audit of Diabetes-Dependent Quality of Life Questionnaire

DOI : 10.20473/jfiki.v8i32021.293-300
Putri Amelia Rooswita , Yunita Nita , Elida Zairina , Gesnita Nugraheni , Libriansyah Libriansyah
293-300
Abstract : 346
PDF : 242

PDF

Login

Username *

Password *

Forgot your password?

Keep me logged in

Login Register

Accredited Sinta II

Instructions

- Guide for Reviewers
- Guide for Authors
- Online Submission
- Document Template
- Copyright Form

Journal Policy

- Focus and Scope
- Publication Ethics
- Article Processing Charge
- Peer Review Process
- Peer Review
- Editorial Team
- Open Access Statement
- Archiving

Plagiarism | Copyright
Contact | Old Website

Publisher

This journal has been published by the Faculty of Pharmacy, Universitas Airlangga in collaboration with IAI of East Java

Meet Our Editorial Team

Elida Zairina, S.Si., MPH., Ph.D., Apt.
Editor in Chief
Universitas Airlangga, Indonesia
-

Suciati, S.Si., MPhil., Ph.D., Apt.
Editorial Board
Universitas Airlangga, Indonesia
-

Susmiandri, S.Kom.
Assistant Editor
Universitas Airlangga, Indonesia
-

Read More

ISSN

9 772406 938003
P-ISSN

9 772580 830001
E-ISSN

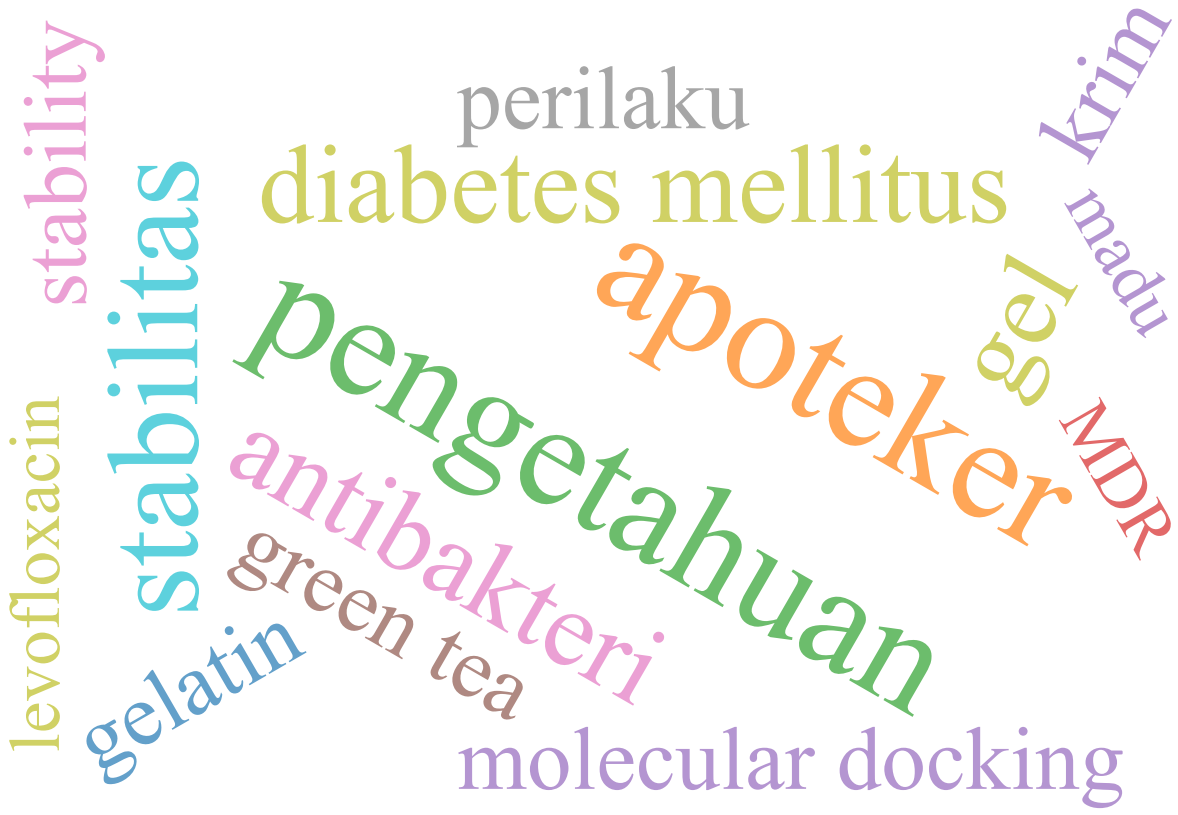
Indexing

GARUDA GARBA RUJUKAN DIGITAL
OCLC WorldCat
Google Scholar
DOAJ DIRECTORY OF OPEN ACCESS JOURNALS
PKP|INDEX
sinta
Dimensions
TREND MD

Visitors

View JFIKI Stats

Keywords



Address

Fakultas Farmasi Universitas Airlangga
Jl. Dr. Ir. H. Soekarno, Mulyorejo, Surabaya,
(Faculty of Pharmacy Universitas Airlangga)
Jawa Timur - 60115, Indonesia

Contact Info:

Telp: 6231-5933150
Fax: 6231-5932549
Email: **jfiki@ff.unair.ac.id**

Lembaga Inovasi, Pengembangan Jurnal, Penerbitan dan Hak Kekayaan Intelektual
LIPJPHKI
Gedung AUP, Kampus C, Universitas Airlangga, Kota Surabaya, Jawa Timur, 60115

The Effect of Serotonin-Norepinephrine Reuptake Inhibitor Milnacipran on Anxiety-like Behaviors in Diabetic Mice

Tuhfatul Ulya[1], Chrismawan Ardianto[2]*, Mahardian Rahmadi[2], Dewi Wara Shinta[2], Junaidi Khotib[2]
[1]Master Program of Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia
[2]Department of Pharmacy Practice, Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

*Corresponding author: chrismawan-a@ff.unair.ac.id

Submitted: 14 September 2020
Accepted: 22 October 2020
Published: 30 November 2021

Abstract

***Background**: Diabetes mellitus is a chronic disease that causes neuronal plasticity and increased hypothalamic pituitary adrenal (HPA) axis of stress disorders. The change in metabolism is reportedly associated with inadequate response to antianxiety and antidepressant agents. **Objective**: This study aimed to determine the effect of milnacipran antidepressants on anxiety-like behavior in mice with diabetes mellitus. **Methods**: Male ICR mice were divided into naive, stress, diabetes mellitus (DM), DM + stress groups to measure anxiety-like behavior. Diabetes mellitus was induced using alloxan, and electric footshock stress was used as a stressor for 14 consecutive days. Anxiety-like behavior was measured using the light-dark box (LDB) and elevated plus maze (EPM) test at days 0, 7 and 14. The antidepressant milnacipran (MIL) was given for 7 days, on days 8 to 14. On day 14, evaluation of anxiety-like behavior after administration of MIL was carried out in all groups using LDB and EPM tests. **Results**: The results showed that administration of milnacipran effectively ameliorated anxiety-like behavior in the non-DM, but not in the DM group, using the LDB test. A similar result was demonstrated in the EPM test showing the non-DM group's attenuation after milnacipran administration. **Conclusion**: The present results indicate that there is an inadequate attenuation of the anxiety-like behavior after treatment with milnacipran in diabetes conditions.*

***Keywords**: antidepressant, anxiety-like behaviors, diabetes mellitus, electric footshock stress, milnacipran*

Abstrak

Pendahuluan: Diabetes melitus merupakan penyakit kronis yang menyebabkan plastisitas saraf dan peningkatan aktivitas *hypothalamic pituitary adrenal (HPA) axis* yang berhubungan dengan gangguan psikologis terkait stres. Gangguan metabolik pada kondisi diabetes dilaporkan memperburuk respon individu dengan gangguan psikologis terkait stres terhadap obat anti-cemas dan antidepresan. **Tujuan**: Penelitian ini bertujuan untuk mengetahui pengaruh pemberian antidepresan milnacipran terhadap perilaku kecemasan mencit diabetes melitus. **Metode**: Mencit ICR jantan dibagi menjadi kelompok naif, stres, diabetes melitus (DM), DM + stres, untuk mengukur *anxiety-like behavior*. Diabetes melitus diinduksi menggunakan aloksan, dan *electric footshock stress* digunakan sebagai stressor selama 14 hari berturut-turut. *Anxiety-like behavior* diukur menggunakan uji *light-dark box (LDB)* dan *elevated plus maze (EPM)* pada hari ke 0, 7, dan 14. Antidepresan milnacipran (MIL) diberikan selama 7 hari, pada hari ke-8 sampai 14. Pada hari ke-14, evaluasi *anxiety-like behavior* setelah pemberian MIL dilakukan pada kelompok naif, stress, stress + MIL, DM + stress, DM + stress + MIL menggunakan uji LDB dan EPM. **Hasil**: Pemberian milnacipran hanya efektif memperbaiki *anxiety-like behavior* pada kelompok non-DM (stres + MIL), tetapi tidak pada kelompok DM dengan uji LDB. Uji EPM menunjukkan hal yang sama, kelompok DM tidak menunjukkan perbaikan *anxiety-like behavior* setelah pemberian milnacipran. **Kesimpulan**: Pemberian antidepresan milnacipran tidak memperbaiki *anxiety-like behavior* pada mencit diabetes mellitus.

Kata kunci: antidepresan, *anxiety-like behavior*, diabetes melitus, *electric footshock stress*, milnacipran

P-ISSN: 2406-9388
E-ISSN: 2580-8303

INTRODUCTION

Diabetes mellitus is a metabolic disease that decreases the quality and life expectancy (Qiu *et al*., 2016). The increasing prevalence of diabetes causes socioeconomic and psychological pressures, a big challenge for individuals (Li *et al*., 2019). Structural and neurophysiological changes in the central nervous system caused by diabetes is associated with cognitive deficits and psychiatric disorders (Myers *et al*., 2013; Qiu *et al*., 2016). In diabetes mellitus, there is a decrease in hippocampal neurogenesis and neuroplasticity changes associated with depression and anxiety disorders (Ho *et al*., 2013).

Depression is a heterogeneous disorder due to changes in monoamine neurotransmitters in the brain, especially norepinephrine (NE) and serotonin (5-HT) (Li *et al*., 2020). Depression as comorbid diabetes was classically introduced 300 years ago (Moulton *et al*., 2015). The likelihood of depression in diabetes mellitus patients ranges from 10 - 15%, two times greater than in the non-diabetic population (Sartorius, 2018). Comorbid depression in diabetes mellitus patients is also associated with poor outcomes. Diabetes mellitus patients with depression have more difficulty controlling blood glucose levels and have unhealthy lifestyles (Li *et al*., 2019). In vivo studies show that depression is determined by measuring experimental animals anxiety levels (Kamei *et al*., 2003).

Improvement of mental conditions, glycemic control and good outcomes is commonly obtained through pharmacological therapy, psychotherapy or a combination of both (Li *et al*., 2019). Various types of drugs that act on the central nervous system have been used clinically as therapeutic agents. However, an effective treatment strategy for anxiety and depression in diabetes mellitus has not been established.

The effectiveness of serotonin noradrenaline reuptake inhibitors (SNRI) on anxiety disorders has been established in clinical trials. However, the therapeutic effect of these drugs varies depending on the type of anxiety disorder (Miyamoto *et al*., 2004). Milnacipran is an SNRI group that simultaneously inhibits 5-HT and NE reuptake (Bourin *et al*., 2005). Murthi & Vaillancourt (2019), in their research, proved that 5-HT might also act as a factor in maintaining normoglycemia. The use of SNRI in the animal study of anxiety-like behaviors showed an increase in the frequency and time of mice entering the open arms (Takeuchi *et al*., 2010). In addition, milnacipran reduces immobility time in stress-induced mice *(Mochizuki et al*., 2002). However, there is a lack of evidence showing the efficacy of milnacipran on the anxiety-like behavior of diabetic mice. Thus, the present study investigated the effect of milnacipran on anxiety-like behaviors in diabetes mellitus.

This study measured anxiety-like behavior after milnacipran treatment in diabetes mellitus mice using the light-dark box (LDB) and elevated plus maze (EPM) tests. These methods are based on the principle that rodents do not prefer to stay in open spaces and heights (Bisong *et al*., 2018). The diabetes mellitus model was developed using alloxan. Alloxan induces diabetes through a partial degradation mechanism of pancreatic beta cells, which affects the quality and quantity of insulin (Ighodaro *et al*., 2017). The electric footshock as a stress stimulus was used to induce anxiety-like behaviors in mice. It is known that many studies effectively produce an adequate anxiety-like state and fear response through inescapable electric foot shock exposure (Silva *et al*., 2020).

MATERIALS AND METHODS

Materials

Milnacipran hydrochloride (Ace Pharmaceuticals, Japan), alloxan monohydrate (Sigma-Aldrich, Germany), citrate buffer pH 4.5 (Sigma-Aldrich).

Tools

Light dark box test apparatus, elevated plus maze test apparatus, electric footshock apparatus, stopwatch, Easy Touch ® blood glucose monitoring system.

Animals

Male (6 - 10 weeks old) ICR mice, weighing between 26 and 30 g, were used. All mice were maintained at a regulated temperature (25°C ± 2°C) and humidity (60 ± 10%) in a 12:12 h diffuse light/dark cycle with free access to food and water. All experiments were performed at the Animal Research Laboratory of the Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia. The design and conduct of the study were in accordance with Helsinki's guidance on animal welfare. All effort was done to reduce the animal number and suffering.

Experimental design and treatments

Mice were divided into naive, stress, diabetes mellitus (DM), and DM + stress groups in experiment I (n = 8). Diabetes mellitus was induced using a single injection of alloxan. An Anxiety-like state was induced by electric footshock stimulus (0.45 mA, 1 s) 30 times with 9-second intervals for 14 consecutive days. Naive mice as the control group received a normal saline

P-ISSN: 2406-9388
E-ISSN: 2580-8303

injection and were placed in the electric chamber for 6 minutes a day without shock stimulus. Evaluation of anxiety-like behavior was measured using the light-dark box (LDB) and elevated plus maze (EPM) test at days 0, 7 and 14. In experiment II (n = 12), an evaluation of anxiety-like behavior after administering milnacipran (MIL) was carried out in the naive, stress, stress + MIL, DM + stress, DM + stress + MIL on day 14. The antidepressant milnacipran at a dose of 20 mg/Kg, reportedly effective in ameliorating anxiety-like behavior (Takeuchi *et al.*, 2010) was given for seven days, on days 8 to 14.

Milnacipran was dissolved in saline and administered orally. The drug was given once a day.

Alloxan-induced diabetes mellitus

Mice were induced with diabetes mellitus by injecting alloxan 170 mg/Kg intraperitoneally (Ighodaro *et al.*, 2017). Alloxan monohydrate was dissolved in a citrate buffer pH 4.5. The blood glucose levels of the mice were checked 48 h after injection with a strip test. Mice with blood glucose levels ≥ 200 mg/dL or 11.1 mmol/L are considered to have diabetes mellitus.

The electric footshock stress procedure

Mice were put into a box measuring 18 x 15 cm with a grid floor from steel,the middle of the box was given a bulkhead. Stress was induced by an inescapable electric footshock with a voltage of 0.45 mA, 60 volts, as previously described (Seo, 2018). A set of electric footshocks has a duration of 1 sec, repeated 30 times at 9-sec intervals. The stress-induced group received two sets of electric footshocks per day. Electric footshocks were exposed for 14 days.

Light dark box (LDB) test

The protocol was performed following the previous study (Zhang *et al.*, 2020). The instrument used consists of two boxes separated by a bulkhead and connected by a door (5 x 5 cm). The light box (27 x 18 x 18 cm) is lit with a white 60-watt incandescent bulb. The dark box (18 x 18 x 18 cm) is completely black. Both are equipped with cameras to record the movements of mice. Mice were placed in a light box facing the door and allowed to explore both boxes for 5 min. The time spent in each box was recorded. The percentage of time spent in the light box was also calculated.

Elevated plus maze (EPM) test

The protocol was performed following the previous study (Walia *et al.*, 2019; Zhang *et al.*, 2020). The apparatus consists of two open and closed arms (each 30 × 6 cm). Closed arm surrounded by 15 cm wall. The arms cover the central area of the maze with a size of 5 × 5 cm. The apparatus is placed 50 cm above the floor. The mice were placed in the centre area, facing the open arms and exploring the arms for 5 minutes. A camera was positioned above the apparatus to record mice activity. The number of entries to the open arm was recorded, and the percentage was calculated. The movement of mice was calculated when all four legs entered the arm.

Statistical analysis

Data are presented as mean ± SEM. Measurement of anxiety-like behavior using the LDB and EPM tests at several time points were analyzed using the two-way ANOVA test, followed by the Bonferroni post-hoc test. Meanwhile, the measurement of anxiety-like behavior after milnacipran administration was analyzed using the one-way ANOVA test, followed by the Bonferroni post hoc test. The difference was considered significant if $p < 0.05$ (95%).

RESULTS AND DISCUSSION

The effect of stress induction on anxiety-like behaviors

This study observed stress-induced diabetes mellitus mice using the electric footshock method with the light-dark box (LDB) and elevated plus maze (EPM) tests. The LDB test showed that the stress group significantly reduced time spent in the light box compared to the naive group, seen on days 7 and 14. In addition, the DM + stress group also showed the same results compared to the DM group (Figure 1).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

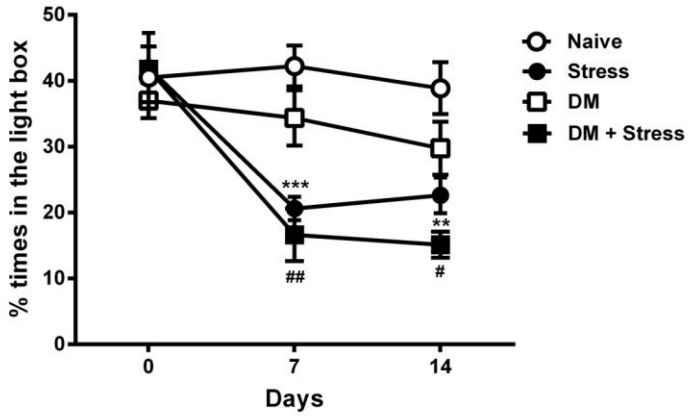


Figure 1. The effect of electric footshock stress induction on anxiety-like behaviors in mice with diabetes mellitus measured by light-dark box test (mean ± SEM) of 8 mice. **p < 0.01, ***p < 0.001 vs naive group. $^{\#}$p < 0.05, $^{\#\#}$p < 0.01 vs DM group. DM, diabetes mellitus

On the other hand, EPM test results showed no difference in the percentage of open-arm exploration between the stress and naive groups. However, a significant difference was found in the DM + stress group, which had a lower percentage of open-arm exploration than the inexperienced group at day 14 (Figure 2).

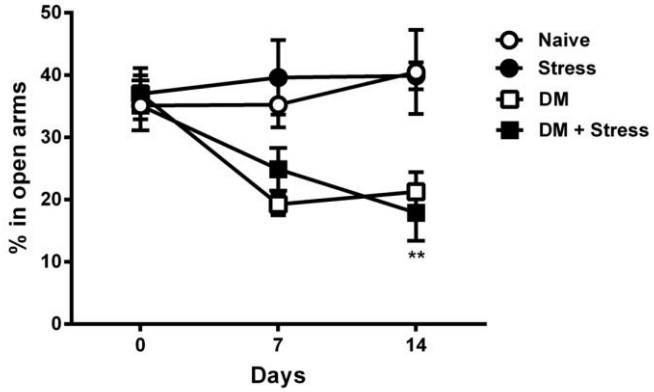


Figure 2. The effect of electric footshock stress induction on anxiety-like behaviors in mice with diabetes mellitus measured by elevated plus-maze test (mean ± SEM) of 8 mice. **p < 0.01 vs naive group. DM, diabetes mellitus

This result suggests that the present method of stress induction successfully induces anxiety-like behavior reflected in LDB, but not the EPM test. This condition might be due to the severity of stress stimulus during installation that may produce a differential anxiety-like response to specific situations, such as light space or open space stimulus. Moreover, it is known that anxiety with complex emotional states may not be generalized as an expression of a single established behavior.

The difference in EPM conditions and equipment was a factor in the different results. Previous studies of stress induction by the electric footshock method in experimental animals 24 and 48 h before measurement showed no difference in the percentage of time spent in mice in open arms (Grahn *et al*., 1995). Another study comparing the effectiveness of test results between elevated zero maze (EZM) and EPM during repeated trials showed behavior in EZM remained relatively stable for several trials and was more suitable than EPM for anxiety experiments (Tucker & McCabe, 2017).

However, the results of the two tests in this study indicate that the condition of diabetes mellitus with stress possibly increases anxiety-like behaviors in experimental animals compared to diabetes mellitus and/or naive conditions alone. This result is in accordance with previous studies that showed an increase in the severity of anxiety in streptozotocin-induced diabetes mellitus mice (Yuan *et al*., 2019).

P-ISSN: 2406-9388
E-ISSN: 2580-8303

The effect of milnacipran treatment against anxiety-like behaviors

The results showed that administration of milnacipran effectively ameliorated anxiety-like behavior measured with the LDB test in the non-DM group (stress + MIL), but not in the DM group (DM + stress + MIL, Figure 3). This result was demonstrated by the increased time spent in the light box only in the stressed group given milnacipran.

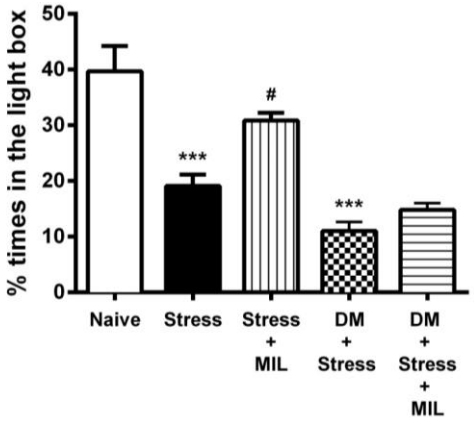


Figure 3. The effect of milnacipran on percentage time spent in the light box in diabetes mellitus mice with anxiety-like behaviors, measured by light-dark box test at day 14 (mean ± SEM) of 12 mice. ***p < 0.001 vs naive group. #p < 0.05 vs stress group. DM, diabetes mellitus; MIL, milnacipran

Similarly, the EPM test results showed that milnacipran did not affect the anxiety-like behavior in the stressed DM group. The present study showed no attenuation in the decreased percentage of open-arm exploration compared to the group without milnacipran administration (Figure 4).

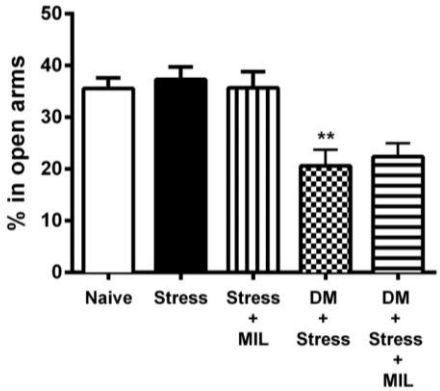


Figure 4. The effect of milnacipran on the percentage of open-arm exploration in diabetes mellitus mice with anxiety-like behaviors was measured by elevated plus maze test at day 14 (mean ± SEM) of 12 mice. **p < 0.01 vs naive group. DM, diabetes mellitus; MIL, milnacipran

Diabetes mellitus has been reported to increase anxiety-like behaviors. Animals with anxious behavior exhibit lower extracellular serotonin levels than normal animals. It is said that decreased serotonin levels is closely associated with anxiety. Furthermore, it is known that there is a change in serotonin activity in diabetic conditions. The animal model for DM exposed to stress stimulus demonstrates lower extracellular serotonin levels in the hypothalamus than those without stress induction and non-diabetic stressed animal (Thorré *et al*., 1997). In addition, serotonin modulates noradrenaline (NA) activity. A decrease in serotonin levels is associated with reducing NA levels (Moret et al., 2011). Milnacipran, an antianxiety drug used in the present study, is an SNRI drug that inhibits serotonin and NA uptake with the same potency, without any affinity for dopaminergic transporters (Bourin *et al*., 2005; Li *et al*., 2020).

The present results show that milnacipran is not effective at attenuating anxiety-like behaviors in diabetes mellitus. The effect of milnacipran may be lowered due to the changes in the brain's serotonin and NA systems. However, it is also possible that increasing the dose of milnacipran may attenuate anxiety-like

P-ISSN: 2406-9388
E-ISSN: 2580-8303

behaviors in diabetes mellitus. The study by Takeuchi *et al.* (2010) showed a dose-dependent of milnacipran increased the time spent in open-arms and the number of open-arm entries. Further research is needed to clarify this issue and the exact mechanisms.

Moreover, previous research has shown a correlation between changes in the brain's glial cells of diabetic mice with anxiety phenotypes. In addition, it was reported that astrocyte activation is found in the hippocampus of diabetic mice, the area that contributes to the development of fear memory and depression (Saravia *et al*., 2002). Studies showed that the marker protein for astrocyte activation, GFAP, is precisely regulated together with the upregulation of IL-6, indicating a neurological inflammatory response in the CNS of diabetic mice (Qiao *et al*., 2016; Yuan *et al.*, 2019). This condition suggests that anxiety states in diabetes mellitus comprisecomplex changes in the neurotransmission system that may affect the efficacy of pharmacological treatment. It remains to be explored whether the different classes of antianxiety demonstrate distinctive tolerability for treating anxiety in diabetic conditions.

CONCLUSION

It is concluded that the antianxiety effect of milnacipran has deteriorated in diabetes mellitus mice. Furthermore, it is suggested that there is a differential anxiety-like response from the EPM and LDB method for anxiety measurement.

ACKNOWLEDGMENT

This work was supported by Ministry of Education, Culture, Research and Technology with PDUPT grant (Dr. Chrismawan Ardianto) and Tahir Foundation Professorship funding in 2020. We thank to Ms. Nadiyah for the excellent technical assistance.

REFERENCES

Bisong, S. A., Nku, C. O., Nwoke, K. U. & Osim, E. E. (2018). Crude Aqueous Leave Extract of *Carica papaya* linn (Pawpaw) Reduced Anxiety and Fear Related Behaviour in cd1 Mice. *European Journal of Pharmaceutical and Medical Research*; *5*; 488–493.

Bourin, M., Masse, F., Dailly, E. & Hascoët, M. (2005). Anxiolytic-like Effect of Milnacipran in the Four-Plate Test in Mice: Mechanism of Action. *Pharmacology Biochemistry and Behavior*; *81*; 645–656.

Grahn, R. E., Kalman, B. A., Brennan, F. X., Watkins, L. R. & Maier, S. F. (1995). The Elevated Plus-Maze is not Sensitive to the Effect of Stressor Controllability in Rats. *Pharmacology, Biochemistry and Behavior*; *52*; 565–570.

Ho, N., Sommers, M. S. & Lucki, I. (2013). Effects of Diabetes on Hippocampal Neurogenesis: Links to Cognition and Depression. *Neuroscience and Biobehavioral Reviews*; *37*; 1346-1362.

Ighodaro, O. M., Adeosun, A. M. & Akinloye, O. A. (2017). Alloxan-Induced Diabetes, a Common Model for Evaluating the Glycemic-Control Potential of Therapeutic Compounds and Plants Extracts in Experimental Studies. *Medicina (Lithuania)*; *53*; 365–374.

Kamei, J., Miyata, S., Morita, K., Saitoh, A. & Takeda, H. (2003). Effects of Selective Serotonin Reuptake Inhibitors on Immobility Time in the Tail Suspension Test in Streptozotocin-Induced Diabetic Mice. *Pharmacology Biochemistry and Behavior*; *75*; 247–254.

Li, H. Q., Chi, S., Dong, Q. & Yu, J. T. (2019). Pharmacotherapeutic Strategies for Managing Comorbid Depression and Diabetes. *Expert Opinion on Pharmacotherapy*; *20*; 1589–1599.

Li, J., Lu, C., Gao, Z., Feng, Y., Luo, H., Lu, T., Sun, X., Hu, J. & Luo, Y. (2020). SNRIs Achieve Faster Antidepressant Effects than SSRIs by Elevating the Concentrations of Dopamine in the Forebrain. *Neuropharmacology*; *177*; 1-11.

Miyamoto, J., Tsuji, M., Takeda, H., Ohzeki, M., Nawa, H. & Matsumiya, T. (2004). Characterization of the Anxiolytic-Like Effects of Fluvoxamine, Milnacipran and Risperidone in Mice Using the Conditioned Fear Stress Paradigm. *European Journal of Pharmacology*; *504*; 97–103.

Mochizuki, D., Tsujita, R., Yamada, S., Kawasaki, K., Otsuka, Y., Hashimoto, S., Hattori, T., Kitamura, Y. & Miki, N. (2002). Neurochemical and Behavioural Characterization of Milnacipran, a Serotonin and Noradrenaline Reuptake Inhibitor in Rats. *Psychopharmacology*; *162*; 323–332.

Moret, C. & Briley, M. (2011). The Importance of Norepinephrine in Depression. *Neuropsychiatric Disease and Treatment*; *7*; 9–13.

Moulton, C. D., Pickup, J. C. & Ismail, K. (2015). The Link between Depression and Diabetes: the Search for Shared Mechanisms. *The Lancet Diabetes and Endocrinology*; *3*; 461–471.

Murthi, P. & Vaillancourt, C. (2019). Placental Serotonin Systems in Pregnancy Metabolic Complications Associated with Maternal Obesity and Gestational Diabetes Mellitus. *BBA-Molecular Basis of Disease*; *1866*; 1-8.

Myers, A. K., Grannemann, B. D., Lingvay, I. & Trivedi, M. H. (2013). Brief Report: Depression and History of Suicide Attempts in Adults with New-Onset Type 2 Diabetes. *Psychoneuroendocrinology*; *38*; 2810–2814.

Qiao, J., Wang, J., Wang, H., Zhang, Y., Zhu, S., Adilijiang, A., Guo, H., Zhang, R., Guo, W., Luo, G., Qiu, Y., Xu, H., Kong, J., Huang, Q. & Li, X. M. (2016). Regulation of Astrocyte Pathology by Fluoxetine Prevents the Deterioration of Alzheimer Phenotypes in an APP/PS1 Mouse Model. *Glia*; *64*; 240–254.

Qiu, Z. K., He, J. L., Liu, X., Zhang, G. H., Zeng, J., Nie, H., Shen, Y. G. & Chen, J. S. (2016). The Antidepressant-Like Activity of AC-5216, a Ligand for 18KDa Translocator Protein (TSPO), in an Animal Model of Diabetes Mellitus. *Scientific Reports*; *6*; 1–13.

Saravia, F. E., Revsin, Y., Gonzalez Deniselle, M. C., Gonzalez, S. L., Roig, P., Lima, A., Homo-Delarche, F. & De Nicola, A. F. (2002). Increased Astrocyte Reactivity in the Hippocampus of Murine Models of Type 1 Diabetes: the Nonobese Diabetic (NOD) and Streptozotocin-Treated Mice. *Brain Research*; *957*; 345–353.

Sartorius, N. (2018). Depression and Diabetes, Translational Research. *Dialogues in Clinical Neuroscience*; *20*; 47–52.

Seo, J. H. (2018). Treadmill Exercise Alleviates Stress-Induced Anxiety-Like Behaviors in Rats. *Journal of Exercise Rehabilitation*; *14*; 724–730.

Silva, A. I., Holanda, V. A. D., Azevedo Neto, J. G., Silva Junior, E. D., Soares-Rachetti, V. P., Calo, G., Ruzza, C. & Gavioli, E. C. (2020). Blockade of NOP Receptor Modulates Anxiety-Related Behaviors in Mice Exposed to Inescapable Stress. *Psychopharmacology*; *237*; 1633–1642.

Takeuchi, T., Owa, T., Nishino, T. & Kamei, C. (2010). Assessing Anxiolytic-Like Effects of Selective Serotonin Reuptake Inhibitors and Serotonin-Noradrenaline Reuptake Inhibitors using the Elevated Plus Maze in Mice. *Methods and Finding in Experimental and Clinical Pharmacology*; *32*; 113–121.

Thorré, K., Chaouloff, F., Sarre, S., Meeusen, R., Ebinger, G. & Michotte, Y. (1997). Differential Effects of Restraint Stress on Hippocampal 5-HT Metabolism and Extracellular Levels of 5-HT in Streptozotocin-Diabetic Rats. *Brain Research*; *772*; 209–216.

Tucker, L. B. & McCabe, J. T. (2017). Behavior of Male and Female C57Bl/6J Mice is More Consistent with Repeated Trials in the Elevated Zero Maze than in the Elevated Plus Maze. *Frontiers in Behavioral Neuroscience*; *11*; 1–8.

Walia, V., Garg, C. & Garg, M. (2019). NO-sGC-cGMP Signaling Influence the Anxiolytic Like Effect of Lithium in Mice in Light and Dark Box and Elevated Plus Maze. *Brain Research*; *1704*; 114–126.

Yuan, P., Zhang, J., Li, L. & Song, Z. (2019). Fluoxetine Attenuated Anxiety-Like Behaviors in Streptozotocin-Induced Diabetic Mice by Mitigating the Inflammation. *Mediators of Inflammation*; *2019*; 1-8.

Zhang, K., Lu, J. & Yao, L. (2020). Involvement of the Dopamine D1 Receptor System in the Anxiolytic Effect of Cedrol in the Elevated Plus Maze and Light–Dark Box Tests. *Journal of Pharmacological Sciences*; *142*; 26–33.

P-ISSN: 2406-9388
E-ISSN: 2580-8303