OBAT: Jurnal Riset Ilmu Farmasi dan Kesehatan
Vol.2, No.3 May 2024
e-ISSN: 3031-0148; p-ISSN: 3031-013X, Hal 200-210
DOI : https://doi.org/10.61132/obat.v2i3.435

Pengaruh Ekstrak Kulit Pisang Kepok (*Musa Paradisiaca* L.) Sebagai Antidepresan Pada Mencit (*Mus Musculus*) Dengan *Acute Restraint Stress*

Dhea Safitri Ramadhani
Program Studi Pendidikan Dokter, Fakultas Kedokteran,
Universitas Airlangga

Maftuchah Rochmanti
Departemen Anatomi, Histologi, dan Farmakologi, Fakultas Kedokteran,
Universitas Airlangga

Erika Vitri Yulianti
Departemen Psikiatri, Fakultas Kedokteran,
Universitas Airlangga

Address: Jl. Prof.DR. Moestopo No.47, Pacar Kembang,
Kec. Tambaksari, Surabaya, Jawa Timur 60132
Corresponding author: dheasrmdhani@gmail.com

Abstract*: Introduction: Banana peel is an organic waste which is known to have various benefits, especially as an antidepressant for mental health. It inspired to conduct a research on various type of banana peels in Indonesia, particularly on Kepok banana which has been widely studied. This research aimed to prove the effect of Kepok banana peel extract (Musa paradisiaca L.) as an antidepressant in mice (Mus musculus) with acute restraint stress.Method: This research used a laboratory experimental design. The male mice were acclimatized for 3 days. Twenty-four mice were then divided evenly into 4 groups. The first group was given banana peel extract (Musa paradisiaca L.) at a dose of 200 mg / kgBW, the second group was given a dose of 400 mg / kgBB, the third group was given a dose of 800 mg / kgBW and the fourth group was given water as control. Each group was given a dose orally for 14 days and ARS depressed induction for 7 hours. Subsequently, mice were treated to assess depression behavior using the tail suspension test (TST) and forced-swim test (FST) to determine the duration of immobility.Result: The result showed that there was a significant difference ($p<0.01$) between the control group and the experimental group, at TST there was a significant difference ($p<0.01$) between two doses of 400 mg / kgBW and 800 mg / kgBW, as well as on the forced-swim test (FST). In addition, there was a significant difference ($p<0.01$) between two doses of 200 mg / kgBW and 800 mg / kgBW, and between two doses of 400 mg / kgBW and 800 mg / kgBW. Conclusion: These result confirmed that Kepok banana peel extract (Musa paradisiaca L.) was an effective antidepressant in reducing immobility duration with acute restraint stress.*

Keywords*: Kepok banana peel extract, antidepressant, mental health, acute restraint stress, forced-swim test, tail suspension test*

Received: April 30, 2024; Accepted: Mei 31, 2024; Published: Mei 31, 2024
* Ricky Annas, rickyannas64@gmail.com

INTRODUCTION

Depression is defined as a neuropsychiatric disorder accompanied by mood swing syndrome and apathy of doing activities leading to affect a person's thoughts, behavior, feelings, and well-being. The appearance of these symptoms may be caused by disruption of the monoaminergic neurotransmission and or antioxidant-oxidant system (Thakare, *et al*., 2016).

In Indonesia, data of depression cases are collected by *Hasil Riset Kesehatan Dasar* (Riskesdas) or research result of basic health of Indonesian Health Ministry in 2018 which shows that people above 15 years old in Indonesia reaches 6.1%. The highest prevalence of depression was found in Central Sulawesi shown by 12.3% and Gorontalo 10.3%.

One of the most supporting factors in anxiety and depression is oxidative stress. Compared to healthy people, those who are depressed have fewer antioxidants causing DNA destruction. Studies have found that significantly elevated serum level of oxidative stress is a biomarker of acute phase depression patients compared to healthy controls (Black, *et al*., 2015).

Accordingly, the researchers came with an idea of innovating depression treatment by focusing the effect of oxidative stress on depression at the systemic, cellular and molecular levels in order to improve strategies in the development of new antidepressants (Xu, *et al*., 2014) that exploited banana peels as the cure.

The previous research proved that banana peel extract could reduce fear and anxiety (Samad, *et al*., 2017). It was found that emotional stress increases oxidative damage and changes the balance of pro-oxidant and antioxidant factors in the brain (Fontella, 2005).

Banana peel can also be used as a natural source of antioxidants and pro-vitamin A for they contain carotenoids, phenols, and amines. Banana peel is also rich in beneficial bioactive compounds (Pereira and Maraschin, 2015).

This was confirmed by Fatemeh, *et al*., in 2012 which tested the antioxidant content of banana peel extract reaching 94.25% at a concentration of 125 μg / ml which was greater than other fruit parts.

Kepok bananas are one of the superior types of domestic bananas in Indonesia. Usually, this type of banana is processed into chips which can increase added value for exports. Banana kepok itself is a superior variety in Indonesia because it has a wide adaptability (Litbang Pertanian, 2003). It has been known previously that banana peels contain high antioxidants so that researcher are interested in examining the potential of kepok banana peels as an antidepressant in mice (*Mus musculus*) with acute restraint stress.

Mice have been frequently used in depression modeling (Stepanichev, *et al*., 2014). The model is Acute Restraint Stress (ARS), a technique to induce depression-like behavior caused by neuronal oxidative damage in mice. The ARS-induced experimental animals showed increased immobility duration in forced swimming test (FST), increased serum corticosterone causes oxidative stress-dependent change in the cerebral cortex and hippocampus, especially increased thiobarbituric acid reactive substances and reduced catalase activity, superoxide dismutase (Thakare, *et al*., 2016).

In this research, a change in immobility behavior of mice was measured in seconds to determine the effect of Kepok banana peel extract (*Musa paradisiaca* L.) as an antidepressant in mice (*Mus musculus*) by induction of acute restraint stress.

MATERIAL AND METHODS

This research was experimental research using a simple randomization method. As many as twenty four, 15-30 gram mice purchased from Pusat Veterina Farma, Surabaya were tested in this research. Mice were grouped into 4, consisting of 1 control group and 3 experimental groups. Each group was caged in 12:12 time division, one group was caged for 12 hours in the dark, and another group was caged for 12 hours in light during the experiment. They had free access to the balanced rodent pellet diet and water. All experiments were approved by The Ethical Committee of Medical Research Faculty of Dentistry, Jember University, Jember, East Java, Indonesia and performed in Biomedical Laboratory of Dentistry Faculty, Jember University.

Bananas Peel Extraction:

The Kepok banana peel extract was purchased from Materia Medica Batu, Batu, East Java, Indonesia and identified as *Musa paradisiaca* L. The technique of making this extract employed maceration method. The sample was washed and dried in a special drying room. And then it was turned into a powder form and ready to be extracted. The solvent used in this research was methanol. The extracted banana peel was evaporated using a rotary evaporator to obtain a thick extract of the Kepok banana peel (*Musa paradisiaca* L.) (Wahyuni, et al., 2019).

Experimental Animals:

The mice were selected randomly but still met the criteria. The sampling was carried out by a simple randomization method due to homogeneous samples; after meeting the inclusion and exclusion criteria. Inclusion criteria: Male mice, age around 8-12 weeks, weight around 15-30 grams, and obtained from the same type and feed. Exclusion criteria: Animals

died during the adaptation period, looked sick including inactivity, bite wounds on the body and liquid stool, they were also stress and marked by a weight loss of more than 10%. Using Federer's formula (1963), from 4 groups, the number of samples in each group must be at least ± 6 experimental animals.

Treatment Schedule:

Before the samples were tested, they were placed in cages and fed once a day for 3 days for acclimatization. After passing the acclimatization process, the experimental mice (*Mus musculus*) were given the extract per day orally for 14 days respectively: (1) Kepok banana peel extract dose 1 (200 mg/kgBW), (2) Kepok banana peel extract dose 2 (400 mg/kgBW), (3) Kepok banana peel extract dose 3 (800 mg/kgBW), and (4) Water for control group. Then the mice were induced by depression-like behavior ARS for 7 hours. The ARS method was performed as described previously by Thakare *et al*. (2017). Forty minutes after ARS, the test was carried out to see the behavior of mice. Each experimental animal was performed using a tail suspension test (TST) for 6 minutes and a Forced-Swim Test for 6 minutes. (Walia, 2016).

Behavioral Analysis:

Tail Suspension Test (TST)

Adhesive tape was affixed to the mice's tail, leaving 2-3 millimeters outside the adhesive tape, and then it was hung on the suspension box by attaching the middle of the adhesive tape to the inner wall of the cage. This experiment was recorded for 6 minutes. The restricted movement of the forefoot without the hind legs' involvement constitutes immobility (Can, *et al*., 2012).

Forced-Swim Test (FST)

During the experiment, mice were forced to swim in a 35 x 22 cm tank filled with water at room temperature (23-25 ° C). Next was to put the mice slowly into the tank and start recording. This experiment lasted for 6 minutes. After the experiment was completed, the mice were removed and dried with drying paper before being put back in their cages (Can, *et al*., 2012). The state of immobility is described as a state of "despair" when experimental animal realizes that escape and surrender are impossible (behavioral despair). The immobility period is shorter when given antidepressants (Porsolt *et al*., 1977).

Table 1.
Treatment for TST and FST

Variable	Group (n=6)	Dosage
TST	Dose 1	200 mg/kgBW
	Dose 2	400 mg/kgBW
	Dose 3	800 mg/kgBW
	Control	Water treated
FST	Dose 1	200 mg/kgBW
	Dose 2	400 mg/kgBW
	Dose 3	800 mg/kgBW
	Control	Water treated

Data Analysis:

The obtained data then analyzed using one way ANOVA test. Moreover, a Post Hoc test was performed with a *P*-value of 0.01 as a significant value.

RESULTS

Twenty-four mice were acclimatized for 3 days. Afterwards, they were divided into 4 groups to be given Kepok banana peel extract (*Musa paradisiaca* L.) consisting of group 1 (dose 200 mg / kgBW), group 2 (dose 400 mg / kgBB), group 3 (dose 800 mg / kgBB), and control group orally for 14 days. On day 15, ARS was performed for 7 hours. Forty minutes after induction, the TST and FST behavior tests were performed, each of them lasted 6 minutes.

The result found that the immobility duration reduced from the tail suspension test (TST) and forced-swim test (FST) by calculating immobility duration in seconds. The duration of immobility in TST was limited to small and fine movements of the forefoot, as well as the absence of movement. The duration of immobility in FST was balancing body movement in order to keep their body float on the water and did not sink.

Table 2.
Table of mean rank duration of immobility in seconds

Variable	Group	Mean Rank
TST	Dose 1	50,5
	Dose 2	77,67
	Dose 3	42
	Control	140,17
FST	Dose 1	139,67
	Dose 2	143,17
	Dose 3	87,17
	Control	199,67

The table above provides information about the average rank for each treatment. The lowest response for both TST and FST came from the dose 3 of experimental groups. While the highest response, both for TST and FST came from control groups that were not given Kepok banana peel extract, but the same induction (ARS) as in experimental groups.

Table 3.
Normality test using the Kolmogorov-Smirnov test

Variable	Statistic	N	*P*-value
TST	0,1	24	0,2
FST	0,114	24	0,2

From the Kolmogorov-Smirnov normality test above, both tests' significance value was more than *p*-value (0,01), meaning equal to 0,2. This shows that the data used in this research were normally distributed.

Table 4.
Homogenity test

Variable	Levene Statistic	*P*-value
TST	5,593	0,496
FST	14,743	0,414

From the table above it can be concluded that the variants of the four experimental groups, for both FST and TST are the same as indicated by the significance values of 0.496 and 0.414 or more than the *p*-value (0.01).

Table 5.
ANOVA test

	Group	Sum of Squares	Mean Square	*P*-value
TST	Between Groups	38029,500	12676,500	0,006
	Within Groups	45328,333	2266,417	
	Total	83357,833		
FST	Between Groups	35498,167	11832,722	0,000
	Within Groups	16061,667	803,083	
	Total	51559,833		

Based on the table, a significance value of 0.006 for FST and 0.000 for TST is obtained. Thus, at an alpha of 0.01 it was found that there was significant difference in response based on the four experimental groups.

Table 6.
Post Hoc test

	Group	Sum of Squares	Mean Square	*P*-value
FST	Dose 1	Dose 2	-3.500	.900
		Dose 3	52.500	.071
		Control	-60.000*	.041
	Dose 2	Dose 1	3.500	.900
		Dosis3	56.000	.055
		Control	-56.500	.053
	Dose 3	Dose 1	-52.500	.071
		Dose 2	-56.000	.055
		Control	-112.500*	.001
	Control	Dose 1	60.000*	.041
		Dose 2	56.500	.053
		Dose 3	112.500*	.001
TST	Dose 1	Dose 2	-27.167	.112
		Dose 3	8.500	.609
		Control	-89.667*	.000
	Dose2	Dose 1	27.167	.112
		Dose 3	35.667*	.041
		Control	-62.500*	.001
	Dose 3	Dose 1	-8.500	.609
		Dose 2	-35.667*	.041
		Control	-98.167*	.000
	Control	Dose 1	89.667*	.000
		Dose 2	62.500*	.001
		Dose 3	98.167*	.000

Based on the sig value. less than alpha (0.01), at TST, there was a difference between the control and experimental groups and there was a difference between the experimental group dose 2 and dose 3 with a significance value of 0.041. At FST, there was a difference between control and the experimental groups, and there was a difference between the experimental group dose 1 and dose 3 with a significance value of 0.071, and the last was at dose 2 and dose 3 with a significance value of 0.055.

DISCUSSION

Role of Kepok Banana Peel Extract *(Musa paradisiaca L.)* as Antidepressant to Mencit *(Mus musculus)* with Acute Restraint Stress on TST

In TST, mice were hung by the tail using a rope to withstand the weight of mice without hurting them. TST was done as an unavoidable picture of stress that reflects the state of despair of mice. For a while, normal mice will try to escape from suspension on their tail

by performing powerful movements such as swinging, after a few minutes of being stationary and immobile or immobility (Castagne, *et al*., 2011).

Immobility in TST was a state of mice that gave up escaping while being hung. This is a common symptom of depression that was found to have sustainable and unavoidable negative stimuli, thus reducing the efforts of depressed people to avoid such negative stimuli (Cryan, *et al*., 2005). Mobility in TST was a response from the treatment of banana peel extract functions as an antidepressant. It is used as an antidepressant since it has effective components of antidepressant-like effects including flavonoids, oligosaccharides, polysaccharides, and others (Tee and Hasan, 2011). Flavonoids themselves are widely known and utilized because of their therapeutic effects on central nervous system disorders, including depression. This is confirmed in the theory of monoamine depression which states that depression has been associated with a decrease in monoamine levels in the synaptic fissures. The main cause of this decrease is metabolic disorders of the monoamine neurotransmitter catecholamine norepinephrine, serotonin and dopamine (German-Ponciano, *et al*., 2018).

It was mentioned in previous research that flavonoids could pass through hematoenchepalic barrier and work in the central nervous system (Hritcu, *et al*., 2017). It explains the role of flavonoids as antioxidants in the body. Flavonoid content in Kepok banana peel extract (*Musa paradisiaca* L.) which acted as an antioxidant caused significant differences in TST result. This was also proved by the statistical test results that have been conducted, from all three doses; 200 mg/kgBW, 400 mg/kgBW, and 800 mg/kgBW which had significant differences compared to the control groups. Mice that were given Kepok banana peel extract struggled harder even in a state of despair with a significant reduction during immobility.

Role of Kepok Banana Peel Extract *(Musa paradisiaca L.)* as Antidepressant to Mencit *(Mus musculus)* with Acute Restraint Stress on FST

An FST or forced swimming test was performed to evaluate the despair and escaping failure of the experimental animal. The active behavior of mice on FST such as struggling and swimming are attempts of escaping, while passive behavior is immobility (Yankelevitch-Yahav, 2015). In research conducted using FST on Selective Serotonin Reuptake Inhibitors (SSRI) antidepressants showed a decrease in the duration of immobility for both chronic and acute depression induction (Yankelevitch-Yahav, 2015).

From statistical test results, it was found that there was a significant reduction in the dosage of 200 mg / kg, 400 mg / kg and 800 mg / kg affected on mice's body weight. This

was in accordance with the theory that there is an effective component in Kepok banana peel extract (*Musa paradisiaca* L.) like antioxidant that functioning as an antidepressant. One of the antioxidants in Kepok banana peel extract *(Musa paradisiaca L.)* is flavonoid which can penetrate the hematoenchepalic barrier and work directly on the brain by reversing the attenuation of monoamine neurotransmitters (Guan and Liu, 2016). Therefore, the increase in monoamine levels can relieve symptoms of depression. This research used mice as experimental animals to see depression symptoms in FST; the increasing immobility duration reflected in passive movement showing the mice giving up escaping. Meanwhile, active movements such as swimming and trying to escape were the responses of the antidepressants in Kepok banana peel extract (*Musa paradisiaca* L.) used in this research.

CONCLUSION

The administration of Kepok banana peel extract (*Musa paradisiaca* L.) proved to have an antidepressant-like effect by reducing the duration of immobility in tail suspension test (TST) and forced-swim test (FST). Thus, it can be concluded that giving Kepok banana peel extract (*Musa paradisiaca* L.) as an antidepressant is effective in reducing the duration of immobility with acute restraint stress.

DAFTAR REFERENSI

Black CN, Bot M, Scheffer PG, Cuijpers P, Penninx BWJH (2015). Is depression associated with increased oxidative stress ? A systematic review and meta-analysis. Psychoneuroendocrinology 51, 164–175

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012). The mouse forced swim test. Journal of visualized experiments 59

Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD (2012). The tail suspension test. Journal of visualized experiments 59

Castagne V, Moser P, Roux S, Porsolt RD (2011). Rodent models of depression: Forced swim and tail suspension behavioral despair tests in rats and mice. Current Protocols in Neuroscience, Chapter 8, Unit 8.10A

Cryan JF, Mombereau C, Vassout A (2005). The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. Neuroscience and Biobehavioral Reviews, 29(4-5), p 571–625

Fatemeh SR, Saifullah R, Abbas FMA, Azhar ME (2012). Total phenolics, flavonoids and antioxidant activity of banana pulp and peel flours: influence of variety and stage of ripenes. International Food Research Journal, 19(3), p 1041-6

Federer WY (1963). Experimental Design, Theory and Application. New York, Mac. Millan, p 544

Fontella FU, Siqueira IR, Vasconcellos AP, Tabajara AS, Netto CA, Dalmaz C (2005). Repeated restraint stress induces oxidative damage in rat hippocampus. Neurochem, 30, p 105-111

Gałecki P, Szemraj J, Bien´kiewicz M, Florkowski A, Gałecka E (2009). Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. Pharmacological Reports, 61(3), p 436-447

German-Ponciano LJ, Rosas-Sánchez, Rivadeneyra-Domínguez GUE, Rodríguez-Landa, JF (2018). Advances in the Preclinical Research of Some Flavonoids as Potential Antidepressant Agents. Scientifica, 2018, Article ID 2963565, p 1-14

Guan LP, Liu BY (2016). Antidepressant-like effects and mechanisms of flavonoids and related analogues. European Journal of Medicinal Chemistry, p 1-20

Hritcu L, et al (2017). Antidepressant Flavonoids and Their Relationship with Oxidative Stress. Oxidative Medicine and Cellular Longevity, 2017, Article ID 5762172, p 1-18

Kementerian Kesehatan (2018). Prevalensi Depresi di Indonesia. Available from https://databoks.katadata.co.id/datapublish/2019/10/09/provinsi-mana-yang-memiliki-angka-depresi-tertinggi.

Litbang Pertanian (2003). Prospek dan Arah Pengembangan Agribisnis Pisang. Available from https://www.litbang.pertanian.go.id/special/publikasi/doc_hortikultura/pisang/pisang-bagian-b.pdf.

Pereira A, Maraschin M (2015). Banana (Musa spp.) from peel to pulp: Ethnopharmacology, source of bioactive compounds and its relevance to human health. Ethnopharmacology, Elsevier, 160, p 149-163

Porsolt R, Le Pichon M, Jalfre M (1997). Depression: a new animal model sensitive to antidepressant treatments. Nature 266, p 730-732

Samad N, Muneer A, Ullah N, Zaman A, Ayaz M, Ahmad I (2017). Banana fruit pulp and peel involved in antianxiety and antidepressant effects while invigorate memory performance in male mice: Possible role of potential antioxidants. Pakistan Journal of Pharmaceutical Sciences, 30, p 989-995

Thakare V, Dhakane VD, Patel BM (2016). Potential antidepressant-like activity of silymarin in the acute restraint stress in mice: Modulation of corticosterone and oxidative stress response in cerebral cortex and hippocampus. Pharmacological Report, 68(5), p 1020-1027

Tee TP, Hassan HA (2011). Antidepressant-Like Activity of Banana Peel Extract in Mice. Am. Med. J, 2, p 59-64

Wahyuni NKDMS, Rita WS, Asih IAA (2019). Aktivitas Antibakteri Ekstrak Kulit Pisang Kepok Kuning (Musa paradisiaca L.) terhadap Bakteri Staphylococcus aureus dan

Escherichia coli serta Penentuan Total Flavonoid dan Fenol dalam Fraksi Aktif. Jurnal Kimia (Journal of Chemistry), 13(1), p 9-15

Walia V (2016). Influence of Stress and Fluoxetine on Immobility Period of Mice in Tail Suspension Test and Forced Swim Test. Asian Journal of Pharmaceutical and Clinical Research, 9(2)

Wang Q, et al (2017). The recent progress in animal models of depression. Neuro-Psychopharmacology and Biological Psychiatry, 77, p 99-109

Xu Y, Wang C, Klabnik J, O'Donnell M (2014). Novel therapeutic targets in depression and anxiety: antioxidants as a candidate treatment. Neuropharmacology, 12(2), p 108-119

Yankelevitch-Yahav R, Franko M, Huly A, Doron R (2015). The forced swim test as a model of depressive-like behavior. Journal of visualized experiments : JoVE, 97, p 1-7