JTL 10 (2) December 2021
Page: 101-111
Contents list available at Jurnal Teknologi Laboratorium

JURNAL TEKNOLOGI LABORATORIUM

Journal Homepage: www.teknolabjournal.com
ISSN 2580-0191(Online) I ISSN 2338 – 5634(Print)

Original Research

OPEN ACCESS

Check for updates

Anti Depression Potential of Papaya Seed Extracts in Wistar Rat Models: *a Study on Body Weights, Blood Glucose, Interleukin-6 and Malondialdehyde Levels with Force Swimming and Tail Suspension Tests*

Devi Ardila [1], **Ali Napiah Nasution** [2], **Fiska Maya Wardhani** [3], **Refi Ikhtiari** [1*]

[1] Department of Biomedical Sciences, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia.
[2] Department of Tropical Medicine, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia.
[3] Department of Medicine, Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia

Abstract: The purpose of this study is to evaluate the potential of papaya seed extract as an antidepressant based on body weight, glucose levels, Interleukin-6, and malondialdehyde levels in treated rats. Force swimming and tail suspension tests have been conducted to measure acute stress and evaluate response behavior. Results showed that the higher concentration of papaya seed extract correlates to higher effectivity on reducing body weight and lowering glucose levels. Dose-response relationships were also observed on the inhibition effect of interleukin-6 and malondialdehyde more effective at higher extract concentrations. The experimental force swimming test and tail suspension test showed the positive effect of extract administration indicated by the shorter immobilization time. Gas chromatography analysis has confirmed the bioactive content in the extract. The main compounds such as; 9-Octadecenoic acid (Z)-methyl ester, Benzyl nitrile, Hexadecanoic acid- methyl ester, and other saponins might be responsible for the antidepressant effects of papaya seed extract. This research encourages further studies on pharmacodynamics, pharmacokinetics, and biomolecules analysis at cellular levels.

Keywords: Antidepression, Papaya seed extract, Saponins, Tail Suspension, Force, Swimming, IL-6, MDA.

INTRODUCTION

Moderate or severe stress can lead to depression[1] and suppress the immune response[2], such as interleukin-6. The IL-6 plays a role in stress reactions and depressive disorders, especially the physical disturbances that accompany depression in the presence of hypothalamic-pituitary-adrenal (HPA) activity[3]. The active transport systems peripherally release cytokines from the blood to the brain. The IL-6 is one of the most investigated cytokines in animal studies of Major Depressive Disorder (MDD). However, genetic studies of IL6 effects in MDD remain controversial. Increased activity of IL-6 causes depression through activation of HPA or by the influence of neurotransmitter metabolism[3,4]. The endocrine and immune systems are integrated through a bidirectional network in which hormones influence immune function, while immune responses are reflected in neuroendocrine changes.They play a role in modulating the response of the HPA at all three levels: hypothalamus, pituitary, and adrenal. Acute effects

Corresponding author.
E-mail address: refiikhtiari@unprimdn.ac.id (Refi Ikhtiari)
DOI: 10.29238/teknolabjournal.v10i2.316
Received 29 September 2021; Received in revised form 21 November 2021; Accepted 26 November 2021

of cytokines are seen on the central nervous system, particularly the hypothalamus[5,6]. Free oxygen radicals increase due to high respiratory oxygen intake and metabolic turnover during stress. During stress, increased energy requirements caused by poor environmental conditions, backbreaking physical work, and psychological trauma (PTSD) require high oxygen intake to meet energy requirements[5]. Prolonged working hours, workload, fatigue, sleep deprivation, psychological trauma resulting oxidative injury indicated by the formation of 8-hydroxy-deoxyguanosine (8-OH-dG) as a biomarker of oxidative DNA damage[5,7].

Herbal medicine as an alternative to cure acute stress has been known for centuries. The active metabolites contained in plant extracts may contribute to the antidepressant effects to recover the symptoms of depression. Bioactive content in plants such as alkaloids, flavonoids, saponins, tannins, and terpenoids have the ability as an antidepressant as good as tricyclic antidepressant drugs, i.e., amitriptyline[8]. Activating neurotrophic factors, such as BDNF will significantly impact neuronal function. Activating neurotransmitter systems, e.g., primarily serotonergic, noradrenergic, and dopaminergic at specific brain regions such as the hippocampus and prefrontal cortex, will activate a long-term chemical communication[8,9].

Several metabolic compounds such as alkaloids, flavonoids, and saponins from papaya seeds have been reported recently. Some studies reported the potential of papaya seed extract as anti-diabetes[10], lowering glucose level[11], hypoglycemic[12], antiobesity[13]. However, a lack of studies reported on the antidepressant effect of papaya seed extracts in rat models. Therefore, here we report an inclusive analysis of the physiological and biomolecular levels of the antidepressant effect of papaya seed extract. This report covers the effect of antidepressants on the body weights, glucose levels, MDA, IL-6 of Wistar rats under treatment with the Forced Swimming Test (FST) and Tail Suspension Test (T.S.). The efficacy of papaya seed extracts has been compared with amitriptyline 0.01 % (w/v) as a positive control.

MATERIAL AND METHOD

2.1. Materials and Tools

Wistar rats 2-3 months, feeding CP 551, amitriptyline 0.01% w/v, aquadest, filter paper, ethanol 96%, papaya seed, IL-6 reagent (EliKine TM Rat IL-6 ELISA Kit) and MDA Bioassay Kit, Glucose ACCU Check, Digital Balancer, Phytochemical tests reagents, and glassware apparatus.

2.1. Methods

Papaya seeds were dried, crushed, weighed 10 g, then heated in an infusion pan, mixed with 100 cc of distilled water. When the temperature reaches 90 ^{o}C let it settle for 15 min, then occasionally stir. Then filter solutions and add enough hot water to the dregs so that the required volume of infusion is 100 ml (10%). Precondition of Wistar rats was provided by feeding, namely CP 551 for one week. Wistar rats were randomly divided into six groups where each group consisted of 5 individuals:

1. Group K = Normal, no stressed, aquadest.
2. Group K- = Negative control, stress, and aquadest.
3. Group K+ = Positive control, stressed, and amitriptyline 0.01% w/v.
4. P1 = Treated with stressed and 10% extract, and amitriptyline 0.01% w/v.
5. P2 = Treated with stressed and 8% extract, and amitriptyline 0.01% w/v.
6. P3 = Treated with stressed and 6% extract, and amitriptyline 0.01% w/v.

The treated groups were placed in a stress inducer illuminated by a 60-watt lamp for 30 min every day. The Force Swimming Test (FST) was conducted by placing the rats in cylindrical tanks partially filled with water, and the immobilization time gets measured[14]. The Tail Suspension Test (TST) was conducted by hanging the rat tails with tape, and the time of each mouse moving get measured[15].

The blood collection from the heart vessels was conducted by dislocation of cervical vertebral followed by surging the chest cavity to collect 3 ml of blood from the heart. The blood was centrifuged for 10 min at 3000-4000 rpm and let precipitate to get serum layer, then put serum in a microtube and stored at -4 ^{o}C. Blood serum was then analyzed by the ELISA method. To check the IL-6, serum was inserted into the wells given with a Streptavidin-HRP and its reagents. Then incubate for 15 min at room temperature and protect from light. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm. If wavelength correction is available, then set to 540 nm or 570 nm. To check the MDA, the precipitated protein was resuspended in 2.5 ml of acetic acid and added by 3 ml of thiobarbituric acid (2g/L in 2 mol/L Na_2SO_4). The solution was entirely mixed by vortex then closed and sealed with parafilm. The lid with the tube was slightly perforated with a needle. The entire solution was incubated at a temperature of 950 C for 45 min later, cooled with running water, then centrifuged at 3500 rpm for 10 min. The supernatant formed was then put into a cuvet, and its absorbance was measured at 532 nm.

Data are presented in mean ± standard deviation (mean ± S.D.). Tests for normality and homogeneity of data were carried out. If the data is normally distributed and homogeneous, then One Way Anova was applied. If the distribution data is not normal and not homogeneous, then Kruskal Wallis was applied, and also the Post Hoc test with α ≤ 0,05 was considered significant.

RESULTS AND DISCUSSION

3.1 Phytochemistry and GCMS Analysis

The content in the papaya seed extract (Carica Papaya L.) only consists of saponin groups. Unfortunately, the alkaloids, flavonoids, tannins, steroids, and triterpenoids were indicated negative by a preliminary screening of phytochemistry tests.

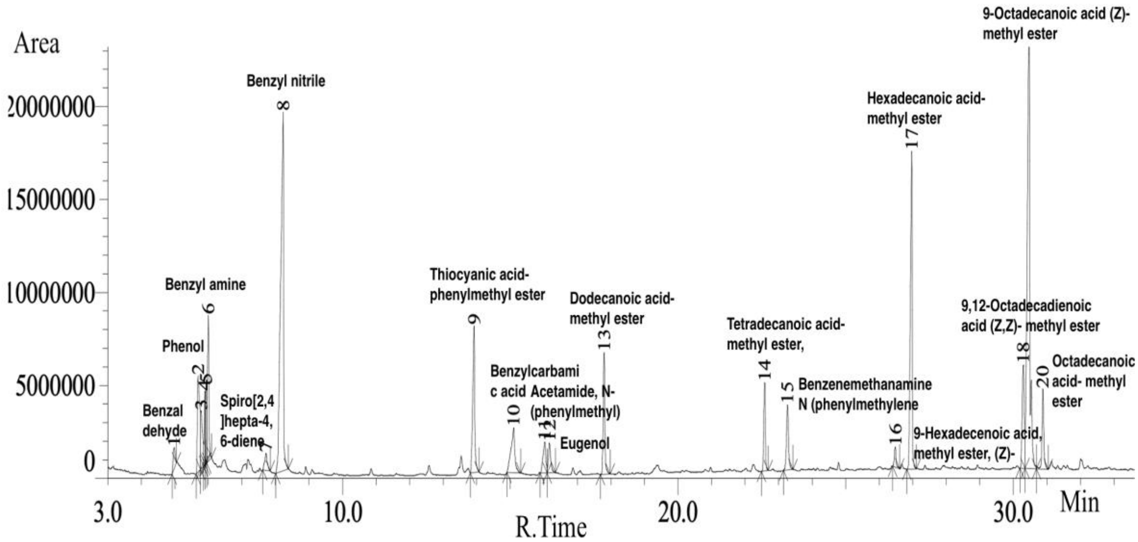

Fig. 1. GCMS Chromatogram of Papaya Seed Extract

The organic compounds in the papaya seeds extract are presented in Figure 1. Based on the peak area (%) of the GCMS data, the main compound was 9-Octadecenoic acid (Z)- methyl ester, a kind of fatty acid methyl ester group, which known as antioxidants[16,5]. Several studies have linked antioxidant effects to phenols and their function as inhibitors of α -glucosides[17,18]. Secondly, Benzyl Nitrile was a precursor for various drugs with potential recreational use[1]. The third primary compound was Hexadecanoic acid-methyl ester, which has antioxidant and anti-inflammatory compound activity[19,20,21]. Generally, plant phenolics are considered vital components of the human diet and exhibit remarkable antioxidant activity and other health benefits[22]. Even though at a small quantity, eugenol was very well known as a component of clove essential oil and exhibited antibacterial, analgesic, anti-inflammatory, and antioxidant properties[23].

3.2 Body Weight Analysis

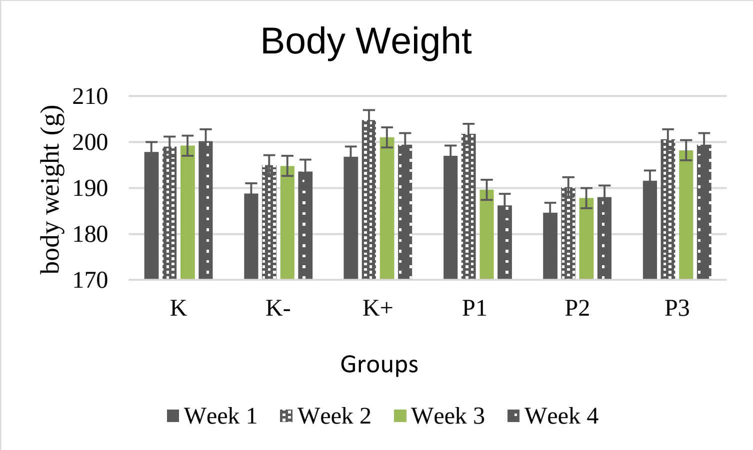

Fig. 2. Body weight of all groups during four weeks of treatment.

Figure 2 shows the differences in body weights of all groups. The body weight decreased significantly in the P1 (186.20 ± 32.136) compared to other groups. Meanwhile, in group K (200.20 ± 47.199), P2 (188.00 ± 23.335) and P3 (199.40 ± 24.966) experienced an increase. There was a decrease for the K+ group (199.40 ± 12.985) but not significant. There was an increase in body weight in the 3rd and 4th weeks of the P2 and P3 groups. However, the weight loss in the P1 group due to the highest concentration of papaya seeds extract at 10%. Where the possibility of the number of active compounds in the content has a more significant effect, based on these data, we can conclude that the higher concentration of papaya seeds extract correlate to higher effectivity on reducing body weight in rat models.

In addition, a specific treatment will reduce the hypothalamic NPY expression and serum leptin in high-fat diet mice[24]. A study showed that chronic stress on repeated Force Swimming Tests reduced body weight and calorie efficiency[25], where the pancreatic lipase enzyme shows an inhibitory effect[13]. While stressed, corticotropin hormone (CRH) will be released from neurons in the parvocellular division[26]. The corticotropin can reduce appetite[23]. Neuropeptide Y also stimulates the inhibition of incoming food (22,23). Overall, stress could have an impact on food intake[25].

3.3 Glucose Levels Analysis

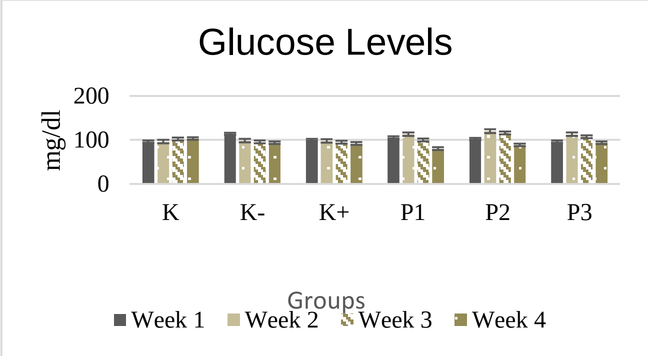

Fig. 3. Glucose levels of all groups during 4 weeks of treatment.

Figure 3 showed a significant effect of papaya seed extract on reducing blood sugar levels of group P1 (80.20 mg/dL ± 15.057). This effect of P1 was better than the P2 (88.80 mg/dL ± 10.86) and P3 (93.60 mg/dL ± 12.661). Papaya seeds contain saponin compounds that have similar activities to insulin. Based on these data, we can conclude that the higher concentration of papaya seed extract correlates to lower glucose levels in the rat.

Saponin could inhibit lipolysis and increase glucose uptake by adipose cells[27]. This study proposed saponins to reduce blood sugar levels with an antihyperglycemic mechanism by stimulating insulin release in pancreatic β-cells[28]. This result was in line with some studies that papaya seed extract could lower blood sugar levels[29,30]. The active substance in papaya seeds may have a hypoglycemic effect that stimulates insulin release from pancreatic beta cells and somatostatin release by suppressing glucagon secretion[10]. In addition, the antioxidant could reduce blood glucose levels by improving pancreatic function (by regenerating cells) to increase insulin production[11]. The main compound, 9-Octadecenoic acid (Z)-, methyl ester, might affect and inhibit the α -glucosidase enzyme activity and minimize the blood glucose level[31].

3.4 Analysis of Interleukin-6

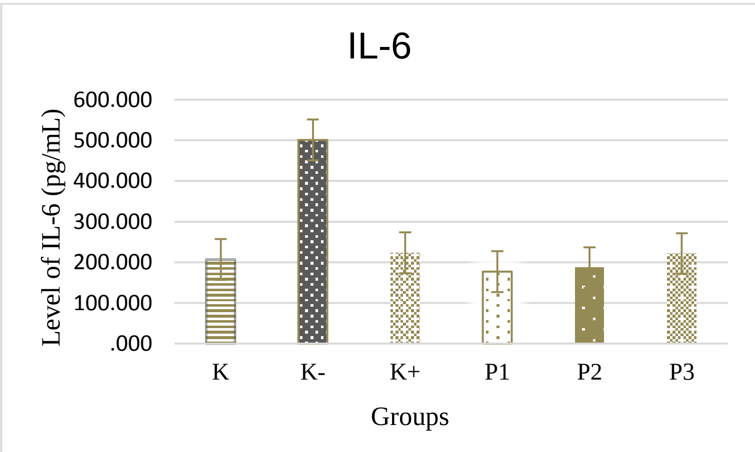

Fig. 4. Level of IL-6 of all groups

Based on the Post Hoc test, the average group was divided into two different groups. The first group is the group of the same average that consists of group P1. The second group consisted of K, K-, K+, P2, and P3. It can be seen that the lowest average of IL-6 levels was P1 (177.3075 pg/ml) and the highest average was K- (500.9739 pg/ml). It was found that the p-value was 0.0489 or

<0.05, which indicated a significant difference in IL-6 between the treatment groups. Based on these data, we can conclude that the higher concentration of papaya seed extract might correlate to lower levels of IL-6.

A study by Nukina et al. (2008) has tested whether stress induces an increase in plasma IL-6 in mice. Plasma IL-6 concentrations increased after one hour of restraint stress and, after that, gradually decreased, indicating that restraint stress could increase plasma IL-6 levels. Further studies found that the induction of increased plasma IL-6 levels by restraint stress was independent of the gut microflora, the primary source of the increase being the liver during stress[32,13]. An increase in IL6 mRNA expression and a fourfold increase in circulating IL-6 levels in the rat hypothalamus was found upon application of restraint stress (3,45). A recent study using prolonged restraint stress in mice also found increased circulating expression of IL 6 mRNA[3,33].

Regarding the metabolite content in the papaya seed extract, we expect that the saponin groups affect T lymphocyte cells in producing cytokinins, thereby suppressing the release of IL-6 activity. While stressed, the IL-6 activity increased, whereas added by plant extract, the IL-6 decreased. In addition, 9-Octadecenoic acid (Z)-, methyl ester may affect IL-6 and play a role in immune cells. However, the process is quite complex, so that more in-depth research is needed to find out. They serve as energy sources and structural components of cell membranes, as signaling molecules and precursors for synthesizing eicosanoids and similar mediators. Another research showed that the localization and organization of fatty acids into different cellular assemblages directly influences the behavior of several proteins involved in immune cell activation, including those related to T cell response, antigen presentation, and fatty acid-derived intermediates as inflammatory agents[34].

3.5 Analysis of Malondialdehyde (MDA)

Figure 5 showed a significantly low MDA level in the P1 group compared to P2 and P3. The positive control (K+) was higher than the P1, with a mean value of 2.2996 ng/ml. Based on the Post Hoc test, it was obtained that the p-value was 0.0464 or < 0.05. This indicated a significant difference in MDA between the treatment groups. Based on these data, we can conclude that the higher concentration of papaya seeds extract might correlate to lower levels of MDA.

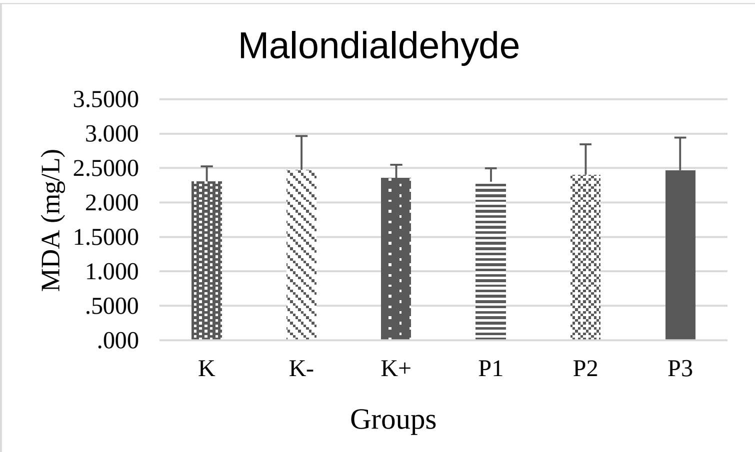

Fig. 5. Level of MDA of all groups

In our study, the compound of 9-Octadecenoic acid (Z)- methyl ester was expected to entrap the free radicals so that MDA lowered. In contrast, the increase of MDA levels was in line with oxidative stress in the body[35]. Papaya seeds have been shown to reduce blood glucose levels and contain flavonoids, alkaloids, and tannins as a source of antioxidants that can entrap free radicals. A study reported the effect of giving papaya seed extract on blood plasma MDA levels in alloxan-induced rats[36]. MDA production was caused by free radicals in the plasma membrane, which causes inflammation. Another study on binahong extract reported an increase in MDA levels at the time of administration caused by the saponin content of binahong. However, this contrasts with another result that showed a decrease in MDA levels in high-dose ethanol extract of binahong leaves, and the causative factors were not known certainly[37].

3.6 Analysis of Forced Swimming Test (FST)

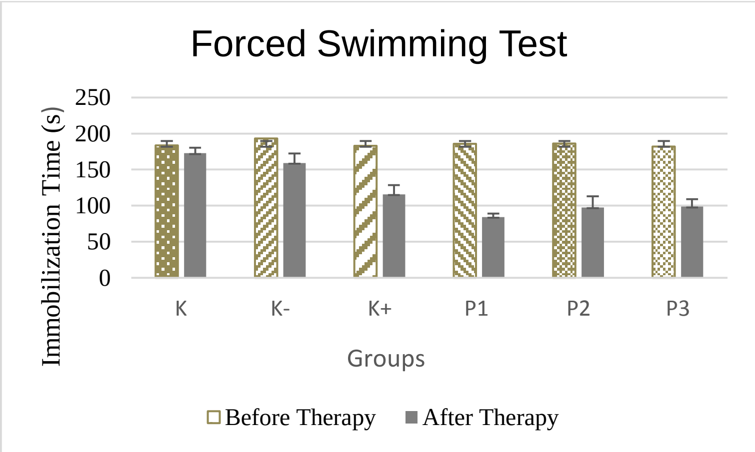

Fig. 6. Forced Swimming Test (FST) of all groups at before and after therapy

The Force Swimming Test showed that the immobilization time in rats decreased significantly in the P1 compared to the P2 and P3 groups. The group was divided into three different groups. The first group was the same average group consisting of P1, P2, and P3. The second group had an average length of FST which consisted of P3 and K+. The third group had the same average length of FST immobilization time consisting of groups K- and K. It was seen that the lowest average FST immobilization was P1=84.20 s and P2=93.60 s. Based on these data, we can conclude that the higher concentration of papaya seeds extract might reduce the immobilization time of FST.

In this study, a group of saponins were found in papaya seeds and might affect neurobehavior. This can be seen from the behavioral test through the FST, where the immobilization time in rats decreased during the FST examination. This occurs because of the involvement of noradrenergic activity[12,35]. Nevertheless, it does not affect the activity of MAO-A and MAO-B[35,38]. In addition, saponins also affect the performance of p-regulation through the BDNF signaling pathway. Where plasma cortisol was decreased, spinal dendritic density was increased, and hippocampal neurogenesis was induced by UCMS[31,35]. Meanwhile, the 5-HT, DA, NE, and 5-HIAA also increased, and the immobilization time decreased[31,38].

3.7 Analysis of Tail Suspension Test (TST)

The Tail Suspension Test showed a significant decrease of immobilization time in group P1 (63.20 s ± 7.791) compared to groups P2 (73.20 s ± 4.658) and

P3 (79.20 s ± 3,633). There was a difference in the negative control group (146.80 s ± 8,167) and the positive control group, where the positive control group (91.20 s ± 5,718) experienced a decrease in TST time more than the negative control group. Based on the Anova test, the calculated F-value was 164,081 with a Sig-value of 0.000. Based on these data, we can conclude that the higher concentration of papaya seeds extract might reduce the immobilization time of TST.

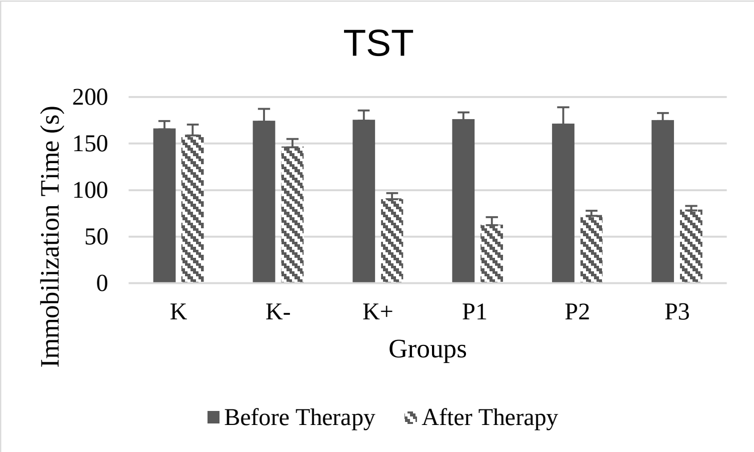

Fig. 7. Tail Suspension Test (TST) of all groups at before and after therapy

Some studies reported that saponin compounds could reduce the immobilization time; meanwhile, the level of 5-HT, DA, and N.E. and 5-HIAA were increased[31,38]. However, there was no effect on MAO-A and MAO-B activities reported[12,38]. Plasma cortisol was decreased, spinal dendritic density was increased, and hippocampal neurogenesis was induced by the UCMS[31,12].

3.7 Proposed Mechanism of Antidepressant

Groups of saponins have been reported to reduce body weight by 20 to 30% by reducing the hypothalamic NPY expression[24]. Neuropeptide Y stimulates the inhibition of incoming food[39,24]. Saponins have insulin-like activity, and they could inhibit lipolysis and increase glucose uptake by adipose cells[29], causing a decrease in blood glucose levels. Saponin compounds also affect neurobehavior. This effect can be seen from the behavioral test through the FST and TST, where the immobilization time in rats decreased during examinations. Some studies reported involvement of noradrenergic activity[38,40]. Here we proposed the mechanism that major compounds and saponin groups contained in papaya seed extract would activate the immune cells to lower IL-6, including those associated with T cell responses. Saponins groups were also suspected of capturing free radicals so that MDA decreased

CONCLUSION

The papaya seeds extract showed potential effects as antidepressants compared to amitriptyline in rat models. The higher concentration of extract might correlate to higher effectivity on reducing body weight, glucose level, IL-6, and MDA, and limited immobilization time of FST and TST. The phytochemical analysis confirmed the main compounds are 9-Octadecenoic acid (Z)-methyl ester, Benzyl nitrile, Hexadecanoic acid- methyl ester, and other saponins contained in the extract might affect the α -glucosidase enzyme to minimize the blood glucose. Additionally, the fatty acids may influence the behavior of several proteins involved in immune cell activation. Further studies on the immune response, enzymatic, hormonal, hypothalamic-pituitary-adrenal, and other neurotrophic factors are also well encouraged.

AUTHORS' CONTRIBUTIONS
All authors contributed equally to this work.

ACKNOWLEDGEMENT
The authors would like to thank the Human Genetic Prima Indonesia University and to all of supervisor.

FOUNDING INFORMATION
Self funding

DATA AVAILABILITY STATEMENT
The utilized data to contribute to this investigation are available from the corresponding author on reasonable request.

DISCLOSURE STATEMENT
The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

REFERENCE

1. Kleiman M, Hawdon J. International Narcotics Control Board. In: *Encyclopedia of Drug Policy*. SAGE Publications, Inc.; 2011. doi:10.4135/9781412976961.n175.
2. Chen M, Singh AK, Repasky EA. Highlighting the Potential for Chronic Stress to Minimize Therapeutic Responses to Radiotherapy through Increased Immunosuppression and Radiation Resistance. *Cancers (Basel)*. 2020;12(12):3853. doi:10.3390/cancers12123853.
3. Burda J, Leszczynski J, Sokalski W. Preface. *Int J Mol Sci*. 2004;5(4):129-129. doi:10.3390/i5040129.
4. Müller N, Ackenheil M. Psychoneuroimmunology and the cytokine action in the CNS: Implications for psychiatric disorders. *Prog Neuro-Psychopharmacology Biol Psychiatry*. 1998;22(1):1-33. doi:10.1016/S0278-5846(97)00179-6.
5. Srivastava KK, Kumar R. Stress, Oxidative Injury and Disease. *Indian J Clin Biochem*. 2015;30(1):3-10. doi:10.1007/s12291-014-0441-5.
6. Dhabhar FS. Immune Cell Distribution, Effects of Stress on. In: *Encyclopedia of Stress*. Elsevier; 2007:449-455. doi:10.1016/B978-012373947-6.00217-8.
7. Irie M, Asami S, Nagata S, Miyata M, Kasai H. Relationships between perceived workload, stress and oxidative DNA damage. *Int Arch Occup Environ Health*. 2001;74(2):153-157. doi:10.1007/s004200000209.
8. Song J, Hou X, Hu X, et al. Not only serotonergic system, but also dopaminergic system involved in albiflorin against chronic unpredictable mild stress-induced depression-like behavior in rats. *Chem Biol Interact*. 2015;242:211-217. doi:10.1016/j.cbi.2015.10.001.
9. German-Ponciano LJ, Rosas-Sánchez GU, Rivadeneyra-Domínguez E, Rodríguez-Landa JF. Advances in the Preclinical Study of Some Flavonoids as Potential Antidepressant Agents. *Scientifica (Cairo)*. 2018;2018:1-14. doi:10.1155/2018/2963565.
10. Rotimi Johnson O, Samuel S, Davou Elnathan W, Hyelni John M. Biochemical effect of Aqueous Carica papaya Seed and Leaf Extracts on

Serum Biochemistry of Alloxan Induced Diabetic Rats. *IOSR J Pharm Biol Sci Ver*. 2015;10(1):2319-7676. www.iosrjournals.org.
11. Puspadewi L. Efek Ekstrak Biji Pepaya (Carica Papaya L .) Terhadap Kadar Glukosa Darah Tikus Putih Jantan (Rattus Novergicus) Galur Wistar Yang Diinduksi Aloksan. *Skripsi*. Universitas Muhamadiyah Surakarta. Published online 2019.
12. Rahman S, Rahmawati R, Nurkholifah N. Efek Hipoglikemik Kombinasi Infusa Biji Alpukat (Persea americana Mill.) dan Biji Pepaya (Carica papaya L Var. Bangkok) Asal Kab. Pinrang Pada Tikus (Rattus norvegicus) Jantan. *Bionature*. 2014;15(02):111-116. doi:10.35580/bionature.v15i2.1557.
13. Od-Ek P, Deenin W, Malakul W, Phoungpetchara I, Tunsophon S. Anti-obesity effect of Carica papaya in high-fat diet fed rats. *Biomed Reports*. 2020;13(4):1-8. doi:10.3892/br.2020.1337.
14. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The Mouse Forced Swim Test. *J Vis Exp*. 2011;58(58):e3638. doi:10.3791/3638.
15. Ang MJ, Lee S, Kim J-C, Kim S-H, Moon C. Behavioral Tasks Evaluating Schizophrenia-like Symptoms in Animal Models: A Recent Update. *Curr Neuropharmacol*. 2021;19(5):641-664. doi:10.2174/1570159X18666200814175114.
16. Rahman MM, Ahmad SH, Mohamed MTM, Ab Rahman MZ. Antimicrobial Compounds from Leaf Extracts of Jatropha curcas , Psidium guajava , and Andrographis paniculata. *Sci World J*. 2014;2014:1-8. doi:10.1155/2014/635240.
17. Belakhdar G, Benjouad A, Abdennebi EH. Determination of some bioactive chemical constituents from Thesium humile Vahl. *J Mater Environ Sci*. 2015;6(10):2778-2783.
18. DJamuna K, Sathiyarajeswaran P, Shree Devi MS, et al. Survival analysis to assess the length of stay of novel coronavirus (COVID-19) patients under Integrated Medicine - Zinc, Vitamin C & Kabasura Kudineer (ZVcKK). *Eur J Mol Clin Med*. 2020;07(10):1375-1387. https://ejmcm.com/article_6680.html.
19. Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *J Basic Microbiol*. 2021;61(6):557-568. doi:10.1002/jobm.202100061.
20. Mazumder K, Nabila A, Aktar A, Farahnaky A. Bioactive Variability and In Vitro and In Vivo Antioxidant Activity of Unprocessed and Processed Flour of Nine Cultivars of Australian lupin Species: A Comprehensive Substantiation. *Antioxidants*. 2020;9(4):282. doi:10.3390/antiox9040282.
21. Siswadi S, Saragih GS. Phytochemical analysis of bioactive compounds in ethanolic extract of Sterculia quadrifida R.Br. In: *AIP Conference Proceedings*. Vol 2353. ; 2021:030098. doi:10.1063/5.0053057.
22. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Reports*. 2019;24:e00370. doi:10.1016/j.btre.2019.e00370.
23. Technology NI of S and. Diacetamide. Published 1988. https://webbook.nist.gov/cgi/cbook.cgi?ID=C625774&Mask=80#IR-Spec.
24. Marrelli M, Conforti F, Araniti F, Statti G. Effects of Saponins on Lipid Metabolism: A Review of Potential Health Benefits in the Treatment of Obesity. *Molecules*. 2016;21(10):1404. doi:10.3390/molecules21101404.
25. Rabasa C, Askevik K, Schéle E, Hu M, Vogel H, Dickson SL. Divergent Metabolic Effects of Acute Versus Chronic Repeated Forced Swim Stress in the Rat. *Obesity*. 2019;27(3):427-433. doi:10.1002/oby.22390.
26. Herman JP, McKlveen JM, Ghosal S, et al. Regulation of the

Hypothalamic-Pituitary-Adrenocortical Stress Response. In: *Comprehensive Physiology*. Vol 6. Wiley; 2016:603-621. doi:10.1002/cphy.c150015.
27. van der Valk ES, Savas M, van Rossum EFC. Stress and Obesity: Are There More Susceptible Individuals? *Curr Obes Rep*. 2018;7(2):193-203. doi:10.1007/s13679-018-0306-y.
28. Harris RBS. Chronic and acute effects of stress on energy balance: are there appropriate animal models? *Am J Physiol Integr Comp Physiol*. 2015;308(4):R250-R265. doi:10.1152/ajpregu.00361.2014.
29. Sudirman S, Kusumastuti AC. Pengaruh Pemberian Rebusan Daun Binahong (Anredera Cordifolia) terhadap Kadar Glukosa Darah pada Wanita Dewasa. *J Nutr Coll*. 2018;7(3):114. doi:10.14710/jnc.v7i3.22270.
30. Haryoto H, Devi ES. Efek Pemberian Ekstrak Etanol Daun Dan Batang Ubi Jalar Ungu (Ipomoea batatas L.) Terhadap Penurunan Kadar Glukosa Darah Pada Tikus Jantan Galur Wistar Yang Diinduksi Aloksan. *Talent Conf Ser Trop Med*. 2018;1(3):139-143. doi:10.32734/tm.v1i3.279.
31. Hidayah K, Kundarto W, Farida Y. Patient Center Care Dalam Penanganan Diabetes Melitus Obese Geriatri Secara Koprehensif. *Annu Pharm Conf*. Published online 2017:108-120.
32. YAO Y, SANG W, YANG X, et al. Antidepressant Effects of Ginsenosides from Panax notoginseng. *J Integr Agric*. 2012;11(3):483-488. doi:10.1016/S2095-3119(12)60034-3.
33. Voorhees JL, Tarr AJ, Wohleb ES, et al. Prolonged Restraint Stress Increases IL-6, Reduces IL-10, and Causes Persistent Depressive-Like Behavior That Is Reversed by Recombinant IL-10. Bartolomucci A, ed. *PLoS One*. 2013;8(3):e58488. doi:10.1371/journal.pone.0058488.
34. Yaqoob P, Calder PC. Fatty acids and immune function: new insights into mechanisms. *Br J Nutr*. 2007;98(S1):S41-S45. doi:10.1017/S0007114507832995.
35. Yang D, Chen X, Liu X, et al. Antioxidant and α-Glucosidase Inhibitory Activities Guided Isolation and Identification of Components from Mango Seed Kernel. Ferretti G, ed. *Oxid Med Cell Longev*. 2020;2020:1-15. doi:10.1155/2020/8858578.
36. Dewi PP. Pengaruh Ekstrak Biji Pepaya (Carica Papaya L.) Terhadap Kadar Malondialdehyde (MDA) Plasma Darah Tikus Jantan Diabetes. *Skripsi*. Univ Islam Sultan Agung Semarang. Published online 2016.
37. Laksmitawati DR, Simbolon R. Aktivitas Ekstrak Daun Binahong(Anredera cordifolia (Ten.) Steenis) sebagai Antihiperurisemia dan Antioksidan pada Tikus Hiperurisemia. *JFIOnline*. 2019;9(1). doi:10.35617/jfi.v9i1.556.
38. Subarnas A, Tadano T, Nakahata N, et al. A possible mechanism of antidepresant activity of beta-amyrin palmitate isolated from lobelia inflata leaves in the forced swimming test. *Life Sci*. 1993;52(3):289-296. doi:10.1016/0024-3205(93)90220-W.
39. National Center for Biotechnology Information Search database. COMPOUND SUMMARY Phenylacetonitrile. National Center for Biotechnology Information.
40. Bahramsoltani R, Farzaei MH, Farahani MS, Rahimi R. Phytochemical constituents as future antidepressants: a comprehensive review. *Rev Neurosci*. 2015;26(6):699-719. doi:10.1515/revneuro-2015-0009.