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Original Article

NEUROPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF AQUEOUS EXTRACT MORINGA OLEIFERA LEAVES

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ABSTRACT

Objective: To investigate the neuroprotective and antioxidant effects of leaves aqueous extract *Moringa oleifera* (MW) in chronic stress mouse models.

Methods: Water immersion and stress restraint for 16 d to obtain a chronic stress model animal. Moringa extract flour dissolved in Aquades, dose 800 mg/kg for 23 d, for chronic Stress+MOW group. Fluoxetine in aquades at a dose of 18 mg/kg BW for 23 d for chronic stress group+Fluoxetine. Aquades were given to normal mice (group N), and mice under chronic stress conditions (chronic stress control group). Furthermore, measure behavioral abnormalities by testing depressive behavior and oxidative stress parameters such as anxiety, Brain-derived neurotrophic factors (BDNF).

Results: Moringa oleifera water extract administration can improve behavioral disorders caused by stress by decreasing immobility time on the Force swim test, increasing time in the middle area, and increasing the number of returns to center areas on the Open field test. When chronically stressed mice were given fluoxetine and MOW, their MDA levels (p=0.008 and 0.041, respectively) and SOD activity (p=0.001 and 0.004) decreased significantly compared to the chronic stress control group. In contrast, Catalase activity increased significantly in chronically stressed mice given fluoxetine and MOW compared to the chronic stress control group (p=0.010 and 0.013). Administration of fluoxetine and MOW may increase the expression of mRNA BDNF compared to the chronic stress control group (p=0.000 and 0.013).

Conclusion: The study found that MOW can improve behavioral abnormalities, namely anxiety and depression behavior caused by chronic stress exposure, through antioxidant pathways and oxidant systems, and also BDNF

Keywords: Moringa oleifera, Anxiety, Oxidative stress, Antioxidant, Chronic stress

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INTRODUCTION

The most widely distributed neurotrophin, brain-derived neurotrophic factor (BDNF), and oxidative stress (OS) may be important in several pathological manifestations of neurodegenerative disorders [1]. The brain-derived neurotrophic factor (BDNF) plays a significant role in various stress-related mood disorders [2]. Stress-related responses have also been altered in animal studies of BDNF deficit *in vivo*, and BDNF is a common downstream intermediary for various exposures that potentiate anxiety-and depressive-like behavior [3].

Chronic stress disrupts prooxidant-antioxidant stability, favoring oxidative reactions. In response to cellular metabolites, ROS causes the creation of peculiar free radical oxidation products [4]. The brain is thought to be particularly vulnerable to oxidative stress or redox imbalances due to its high oxygen consumption and an environment rich in lipids. Thus, it should be no surprise that oxidative stress is linked to a number of brain disorders, including neurodegenerative diseases, psychiatric conditions, and anxiety [5].

Previous literature has demonstrated *M. oleifera* positive benefits, including neuroprotective and antioxidant properties [6]. *Moringa oleifera* leaves are enriched in phytochemicals such as tannins, sterols, saponins, terpenoids, phenolics, alkaloids, and flavonoids [7]. Previous rodent studies found that extracts of *Moringa oleifera* leaves have anxiolytic and antiepileptic properties [8, 9], antidepressant [10], and improves memory [11].

MATERIALS AND METHODS

Materials

The Moringa leaves powder was obtained from PT Javaplant (Solo, Indonesia); it was made by an aqueous extraction method and filled in with maltodextrin. Superoxide Dismutase (SOD) Colorimetric Activity Kit (ab65354, colorimetry, Abcam, Cambridge, UK), catalase activity assay kit (colorimetric, ab83464, Abcam, Cambridge, UK), and Malondialdehyde Lipid Peroxidation (MDA) Assay Kit (MyBiosource, Inc. San Diego, USA, 822354).

Animal preparation

We purchased 24 male BALB/c mice (25-30 g) from Biopharma Laboratory and Animal Breeding, Bandung-Indonesia. The mice were kept in cages in a closed system at 24±2 °C and a 12 h light/dark cycle and allowed access to food and drink *ad libitum*. The rats were randomly divided into four groups of five. Group Normal: normal mice received aquadest. Group stress chronic control: mice with induction of water immersion and restraint stress. Group stress chronic+fluoxetine: mice models chronic stress+administration of fluoxetine (18 mg/kg BW/d/oral) Group stress chronic+MOW: stress chronic mice models+administration of Moringa water extract 800 mg/kg BW/d/oral (MOW). Administration of fluoxetine and Moringa for 23 d of the experiment.

Stress protocol

The combination of water immersion and restraint stress for 16 d was used in this study to create a stress animal model. The combination of restraint stress and water immersion stress, combining psychological and physical stressors, can further cause depressive symptoms [12]. Chronic stress uses the method described by Yasugaki *et al.*, with some modifications. Mice are inserted into a 50 ml conical polypropylene centrifuge tube with several holes for air circulation [10]. Moreover, the tube is immersed in water (the temperature at 22 °C) in a vertical position up to the xiphoid limit.

Behavior test

Open field test for anxiety-like behavior measurement

Anxiety behaviors in the new environment were measured using OFT [13]. The OFT protocol was carried out base on the explanation of Seibenhener, M. L. *et al.*, with some modifications [14]. The tool used on the OFT, measuring 40 cm in length and height, and 160 cm in width, of acrylic material. The observation was recorded for 10 min with a video camera. Anxiety levels are calculated by recording the time spent in the central area (TCA) and the number of trips back to the center (NRC).

Forced swim test for depressive-like behavior measurement

The forced swim test (FST) is used to examine depression-like behavior in research mice. Yankelevitch-Yahav, R. *et al.* report that various adjustments to this procedure were made [15]. We used a set of 15 cm wide by 25 cm high transparent cylindrical glass containers for our experiments [16]. The immobility time parameter (in seconds) is observed and recorded for 4 min of observation. The time of immobility is when the animal is motionless or simply moves to keep its head above the water, and it describes a sign of behavioral despair as representing a depressive response [17].

Biochemical analysis

Rats are sacrificed after 23 d of treatment. The brain tissue is taken washed with cold buffer salt, and preserved at-80 °C.

Tissue preparation

Protein levels, SOD, MDA, and catalase are examined from the prefrontal cortex. Tissue samples were homogenized in an ice-cold 0.1 M phosphate buffer using a homogenizer (pH 7.4). Protein levels were examined with commercial Coomassie Plus (Bradford) assays on a spectrophotometer at 595 nm (Merck B6916) [18].

The Wills method was used to evaluate the lipid peroxidation of the sample tissue. MDA-level measurements were performed based on protocols from the kit manufacturer. The MDA level of each sample is determined based on its protein content and is expressed in nmol/mg protein.

SOD activity in the network is measured based on the manufacturer's protocol of the kit. In summary, the degree of inhibition (%) of the production of water-soluble formazan from water-soluble tetrazolium and superoxide anions produced by the xanthine-xanthine oxidase system, which can be detected by increased absorbance at 450 nm.

Catalase activity measured based on catalase activity kit procedure, Cambridge, UK). Measure on a microplate reader. Output is measured immediately at 570 nm OD on microplate readers, and catalase units are nmol/min/ml or mU/ml.

mRNA BDNF expression of the prefrontal cortex

Total RNA extracted from cortex prefrontal tissue samples was extracted to measure total RNA using a total RNA mini-kit (Geneaid) according to the manufacturer's instructions. Total RNA was measured on a NanoDrop spectrophotometer (BioDrop, UK) at a wavelength of 260 nm. The ReverTra® qPCR RT Master Mix/gDNA removal kit was used in the qRT-PCR investigation of BDNF mRNA expression (Toyobo Bio-Tech).

BDNF-NeuroD1; forward 5'AAGCCATGAATGCAGAGGAGGACT-3';

reverse: 5'AGCTGCAGGCAGCCGGACC'3; and

GAPDH, Forward: 5'-TGCACCACCAACTGCTTAGC-3', and

reverse 5'-GGCATGGACTGTGGTCATGAG-3', were used as primary probes to identify the presence of BDNF and GAPDH[19]. Primer was designed and manufactured by Integrated DNA Technologies, Inc. (Coralville, IA, United States). The $2^{-\Delta\Delta}$CT method was used to evaluate the relative expression level.

Statistical analysis

The mean and standard error of the mean (SEM) results were displayed. A one-way analysis of variance and a post-hoc analysis utilizing the least significant difference test were used to assess the effects. A p-value of greater than 0.05 was used to determine statistical significance.

RESULTS AND DISCUSSION

Effect of MOW on behavior abnormalities

In this study, we performed behavior tests such as anxiety-like behavior in OFT and depression-like behavior in FST. The chronic stress condition reduced the number of TCA and NRC on OFT when compared to the control group (p=0.005 and 0.002). The administration of fluoxetine in stress mice is the same as in the control group. MW 800 mg/kg, in contrast, can significantly (p=0.01 and 0.001) enhance NRC and TCA when compared to the WIRS group. (fig. 1 a, b).

The FST is based on the assumption that when an animal is placed in a container filled with water, it will initially try to escape but will eventually exhibit immobility, which may be interpreted as a sign of behavioral despair [20]. Chronic Stress condition increased in immobility time in FST to the WIRS group (p=0.003). Administration of fluoxetine and MW 800 mg/kg BW showed a significant decrease in immobility time compared to the chronic stress group (p=0.000 and 0.013) (fig. 1c).

The results showed that mice in stressful conditions only increased their anxiety index. This result is evidenced by a decrease in NRC and TRC in OFT, which are in line with Moreno-Martinez *et al.* [21]. Furthermore, we found an increase in immobility time and a decrease in mobility time in FST. Previous studies demonstrated that rats with a history of adolescent stress spent significantly more immobility time than subjects who were not stressed [22].

In the present study, it was found that administration of MOW can improve abnormal behaviors, such as depressive-like behavior and anxiety-like behavior, which are caused by chronic stress exposure. These results align with previous research; significant antiepileptic and anxiolytic effects were seen with Aqueous Extract *M. oleifer*a (250, 375, and 500 mg/kg, i. p.) [8]. Mahmoud, M. S, *et al.*, also reported *M. oleifera l*eaf extract attenuated CCl4-induced anxiety and behavioral changes such as depression [23].

Effect of MOW on oxidative stress parameters

There was a significant difference between the groups, according to the findings of the ANOVA test on the MDA level (p=0.014). It was discovered from the outcomes of the post hoc LSD test that MDA levels considerably increased in the stress control group compared to the normal group (p = 0.003). When fluoxetine and MOW were administered to chronically stressed mice, their MDA levels significantly decreased compared to the chronic stress control group (p=0.008 and 0.041, respectively).

According to the results of the ANOVA test on SOD activity in this study, there was a significant difference between the groups (p=0.001). The post hoc LSD test revealed that SOD activity significantly increased in the stress control group compared to the normal group (p = 0.00), which was another important finding. Chronically stressed mice received MOW and fluoxetine, which significantly reduced their SOD activity compared to the chronic stress control group (p=0.001 and 0.004, respectively).

The results of the ANOVA test on catalase activity showed a significant difference between groups (p=0.001). Furthermore, from the results of the post hoc LSD test, it was found that catalase activity decreased significantly in the stress control group compared to the normal group (p=0.000). Catalase activity in chronically stressed mice that received fluoxetine and MOW experienced a significant increase compared to the chronic stress control group (p=0.010 and 0.013, respectively) (table 1).

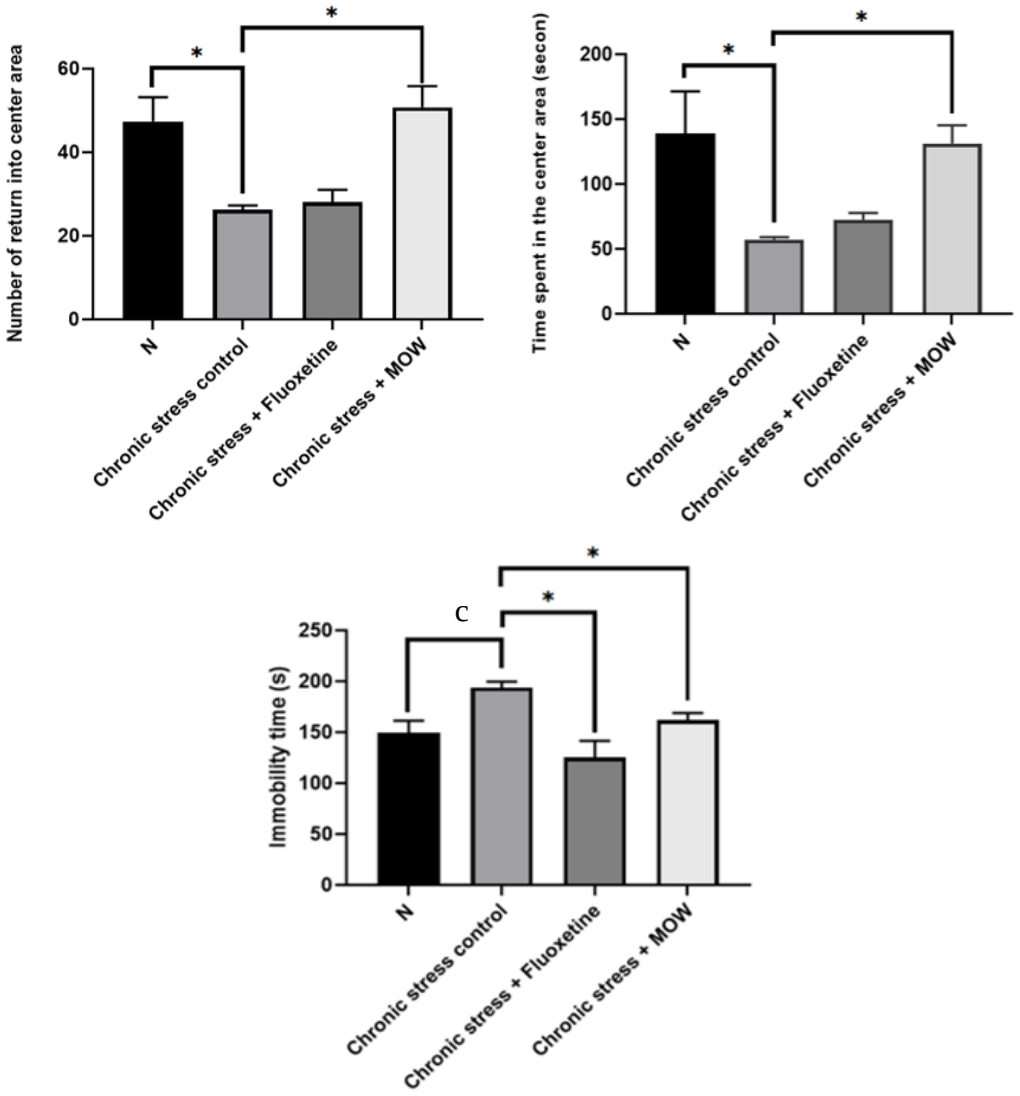

Fig. 1: a. Number of returns into center area (TCA), b. Time spent in the center area (second), c. Immobility time (second). The result is express as a mean±SEM value (n= 6) *p<0.005 compare stress chronic control group

Table 1: Effect of MOW on MDA level, SOD, and catalase activity (n= 3)

Group	MDA level (nmol/mg protein)	SOD activity (U/mg protein)	Catalase activity (nmol/min/mg)
Normal	0.443±0.032*	0.065±0.007*	22.23±1.264*
Stress chronic control	0.800±0.064	0.356±0.057	6.460±1.529
Stress chronic+fluoxetine	0.493±0.044*	0.098±0.003*	13.49±1.017*
Stress chronic+MOW	0.590±0.088*	0.158±0.036*	13.11±1.964*

The result is expressed as a mean±SEM value. *p<0.005 compare stress chronic control group

This study shows chronic stress conditions can increase MDA levels and SOD activity but decrease catalase activity. Numerous studies have shown that people with depression had elevated MDA serum levels [24, 25]. Furthermore, in the schizophrenia group, elevated SOD levels correlated positively with subscores of general psychopathology and negative symptoms [26]. Whereas catalase deficiency has been linked to various diseases, including Alzheimer's disease, bipolar disorder, and schizophrenia, as well as systemic disorders [27].

While MOW is on pressing MDA levels and SOD activity, while in catalase activity, MOW increases its activity. Lamoub, *et al.* report this aqueous extract of *Moringa oleifera* also increases the activity of antioxidant enzymes and reduces levels of malondialdehyde in the blood [28]. Our previous study found MOW has a compound of triterpenoid, polyphenolic, saponin, tannin, flavonoid, alkaloid and anthraquinone [11]. Flavonoid is a secondary metabolite that can activate antioxidant enzymes and reduce lipid peroxidation [29]. Notably, the flavonoids and polyphenolics contained in MOE, including quercetin and rutin, are recognized to have antioxidant and neuroprotective effects [30]. It may be possible to increase glutathione redox equilibrium and the enzyme activity of CAT, SOD, glutathione peroxidase (GPx), and transferase by supplementing with *Moringa oleifera* leaf extract to prevent lipid peroxidation and oxidative damage (GST) [31].

Effect of MOW in mRNA BDNF expression

Measurement of BDNF mRNA expression levels was performed to determine the effect of MOW on synaptic plasticity. Fig. 2 shows

mice in the stress control group experienced a significant decrease in the mRNA expression level of BDNF (p=0.003) compared to the normal group; in contrats, administration of fluoxetine and MOW may increase expression levels compared to the chronic stress control group (p=0.000 and 0.013). A raise in BDNF mRNA expression in the hippocampal and cortical regions parallels the antidepressant-like response of standard antidepressant medications such as SSRIs [32]. Antidepressant therapy increases BDNF activity as well as several types of neuronal plasticity, such as neurogenesis, synaptogenesis, and neuronal maturation [33]. Previously, animals were given a combined dose of 200 mg/kg/d MOE+10 mg/kg/d fluoxetine for 14 d, which produced antidepressant effects. Such antidepressant effects are achieved via the noradrenergic-serotonergic neurotransmission pathway, which is typical of drug-class selective serotonin reuptake inhibitors (SSRIs) [34]. In research, it was found that MOW has the potential as an antidepressant by increasing the expression of BDNF.

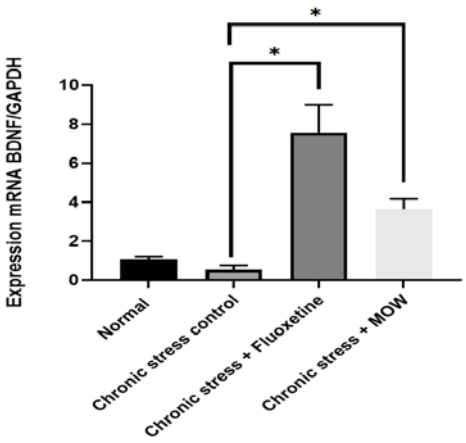

Fig. 2: Expression mRNA BDNF/GAPDH. The result is expressed as a mean±SEM value. (n=3); *p<0.005 compare the chronic stress control group

CONCLUSION

The study found that MOW can improve behavioral abnormalities, namely anxiety and depression behavior caused by chronic stress exposure, through antioxidant pathways and oxidant systems, and also BDNF.

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AUTHORS CONTRIBUTIONS

All authors have contributed fairly.

CONFLICT OF INTERESTS

Among the authors have no conflict of interest

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