The Effect of Moringa Oleifera Leaves Powder to Level of Serum Superoxide Dismutase (SOD), Lead (Pb), Zink (Zn) and Memory Function of Rat (Rattus norvegicus) Wistar Strain Model of Autism that is Exposed by Pb

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Abstract Lead exposure (Pb) may aggravate the decrease in serum SOD levels, memory function reduction and social interaction in autism. This study aims to determine the effect of Moringa oleifera on autism models of rat exposed to lead. The five groups of rat exposed to tin were studied: normal rat (C -), autistic rat (C +) and autism rats given 180 mg (T1), 360 mg (T2) or 720 mg (T3) oleifera leaves powder. Lead acetate is given through a filler tube (0.5 gr / kg) and powdered moringa leaves mixed with feed. The results showed that serum SOD levels were lower in autistic rat than normal rat and intake of Moringa oleifera leaves powder increased serum SOD levels in autistic rats but was not seen in normal rat. The autistic rat had higher lead levels but lower zinc levels, which were not influenced by Moringa oleifera. The social interaction between rats is strongly influenced by unbidden lead with Moringa oleifera, both in normal rat and in autistic rat. Moringa oleifera will increase serum SOD levels in autistic rats but not at normal levels. Moringa oleifera does not protect significantly rat from lead causing less social interaction, nor does it lower serum Pb levels or increase Zn levels. Moringa leaves powder does not affect memory significantly.

Introduction Autism is a neurological disorder with limitations on social interaction, communication, hyperactive behavior, and intellectual (American Psychiatric, 2013). The prevalence of autism increases annually with an increase of 0.84% in 2012 to 2% in 2007 (Blumberg et al., 2013). Based on the results of the US Census Bureau in 2005, people with autism in Indonesia reached 475,000 people (Mahardhika & Budiono, 2012).

The risk of autism is five times higher in boys (Christensen et al., 2016). Combined internal and external factors lead to greater risk of autism. Impaired regulation of neurological function due to brain abnormalities (Rahayu, 2014), genes (Chaste & Leboyer, 2012), and damage to the amygdala can cause disruption to the delivery of information to the brain, impaired expression and emotion (Schultz, 2005). They cause fear, anxiety, and social behavior disorders (Wang et al., 2013). Previous research has shown that the distribution of neuronal disorders in sodium valproate-induced rats (NaVPA) causes autism symptoms such as limited social interactions (Wang et al., 2013) (Chen et al., 2014) and hyperactivity (Markram et al., 2008).

The state of autism can be exacerbated by heavy metal contamination. This is because...
most children with autism have metallothionein dysfunction, an endogenous protein that acts as a heavy metal binder into the body (Bjørklund, 2013). One of the many heavy metals found in the environment is tin. Lead or heavy metal is a neurotoxin that affects brain development so susceptible to damage. Lead poisoning in children can cause negative effects on the brain that function for cognitive, communication, and social. Previous research has shown that children with a history of lead poisoning during developmental periods exhibit symptoms such as autism (Lidsky & Schneider, 2005). The heavy metals accumulated in the body will cause ROS (Reactive Oxygen Species) to inactivate enzymes such as superoxide dismutase (SOD) and Glutathione Peroxidase (GOPD), which function as antioxidants (Birben et al., 2012). Decreased levels of antioxidants will also increase oxidative stress that may cause cognitive and memory dysfunction (Farr et al., 2003). Treatment with antioxidants can improve cognition and memory impairment (La Fata, Weber, & Mohajeri, 2014).

One way to resist free radicals in the body is to consume foods that contain high antioxidants. Moringa oleifera is a natural source of antioxidants containing phenolics, polyphenols oxidase, phenolic acids and flavonoids (quercetin, kaempferol). Based on the above, we undertook research relating to the effects of olifera olefera leaves powder to serum SOD and memory function of autism rat models exposed to acetate lead.

Research Methods

Research design.

This research has been approved by the ethics team of Bioscience Laboratory and also conducted in a physiology laboratory, University of Brawijaya. This study was an in vivo laboratory experiment with a simple experimental design with Post Treatment only One Controlled Group Design. The independent variables in this study were autism model rat given by olifera leaves powder with multilevel dose (180 mg, 360 mg, and 720 mg) and the dependent variable of this study was Pb and Zn blood level, serum SOD and memory.

Population and Sample.

The population in this study was the male autism model wistar strain rat (Rattus norvegicus). The samples used male rats (Rattus norvegicus) Wistar strain of 20 rats were divided into 5 groups.

Tools and materials.

Analytical balance, Y pipe, syringe, sonde, cage, stopwatch, scalpel, eppendof tube, micropipette, centrifugal, spectrophotometry, mini polypropylene pool and footing place, wooden maze, cotton bud, microscope, thermometer, and meter, single intraperitoneal 600 mg/kg NaVPA, lead acetate, moringa leaves powder, powder (pellet), distilled water, flour, methylene blue 0.1% alcohol, 95% NaCl, and reagents (assay buffer, sample buffer, radical detector, standard SOD, xanthine oxidase).

Determination of Treatment.

Autism model rats from offspring of female rats which were in the estrus phase mated with male rats. Estrus phase is seen using a vaginal swab stained with methylene blue showed cornified cells. At 12.5 days gestation, female rats were injected with NaVPA 600 mg/kg. Male offspring rat were weaned after 26 days for adaptation stage before treatment. Treatment in the sample was performed for 14 days with acetate lead exposure of 0.5 g/kg. There were five groups of subjects treated in the study: Group 1. Negative Control: normal group of rat exposed by lead-acetate for 14 days, Group 2. Positive control: autism model rat group exposed by lead-acetate for 14 days, Group 3 autism model rat-exposed group by lead-acetate and 180 mg of olifera leaves powder for 14 days, Group 4. autism model rat group exposed by lead-acetate for 14 days 360 ml of
Moringa leaves for 14 days, Group 5. autism model rat group exposed by lead-acetate for 14 days and 720 mg of Moringa oleifera for 14 days.

Lead acetate was administered through a filler tube (0.5 gr/kg) and the Moringa oleifera leaves powder was mixed with feed. Feeding is done 2 times a day with each giving 15 gr.

**SOD Serum Level Test**

Blood sampling is performed through cardiac surgery when inserted into the test tube without anticoagulation. It is stored at 25°C for 30 minutes. Then the blood is centrifuged at 2000 rpm for 15 minutes. Serum taken with micropipette, then inserted into eppendorf tube (Company, 2010). Ten mililiter serum samples were inserted into small test tubes, plus 90 mL of distilled water, 0.0518M 2775 mL carbonate buffer, and 1250 mL 0.01 M epinephrine solution. Then the mixture was homogenized, fed into cuvette, and measured by absorbance at minutes 1, 2, 3 and 4 at a wavelength of 480 nm and a temperature of 30°C using Shimadzu UV-visible spectrophotometer UV-1601 spectrophotometer at a wavelength (580 nm).

**Test Memory**

Memory tests were performed using the Water Morris maze test that has been modified. Rat are placed in pools made of polypropylene and are made aisle directed to the foothold. The time in reaching the footing is recorded as a result of rat memory. The diameter of the swimming pool is 86 cm with a height of 25 cm. Swimming pool water with 15 cm height mixed with flour and platform rectangle placed 2 cm under water. Water temperature ranges from 25°C.

The modified test is done for 3 days with 3 repetitions. The rats were trained to find a platform that is 2cm below the surface of the water. The time has ended when the rats rest on the platform for 2 seconds or after swimming for 60 seconds, but have not reached the platform yet. If the rat fails to find the platform for 60 seconds, the rat will be guided to find the platform and placed on the platform for 20 seconds before the next exercise. The time rat reached the recorded platform.

**Measurement of Zn and Pb levels in the blood.**

Measurement of blood Zn and Pb levels was performed using Atom Absorbance Spectofotometry (AAS).

**Data Collection and Data Analysis.**

The SOD test used spectrophotometric methods and rat memory function tests using the modified Morris Water Maze on day 14 after treatment.

**Result and Discussions**

**Feed Intake**

Based on tests of homogeneity and normality average intake of rats during the treatment is homogenous and normally distributed with p values are respectively 0.59 and 0.264. Based One Way ANOVA test was obtained p>0.05 (p = 0.174), which means there are no significant differences in feed intake between groups.

| Group               | feed (%) | Moringa oleifera leaves powder (mg) |
|---------------------|----------|------------------------------------|
| Negative Control    | 73.50    | -                                  |
| Positive Control    | 66.43    | -                                  |
| Treatment 1         | 67.05    | 120.69                             |
| Treatment 2         | 75.42    | 271.51                             |
| Treatment 3         | 63.19    | 454.97                             |

**Social interaction**

To analyze the social interaction test before being given Independent t-test between groups of children with autistic rat and normal rat was different with p<0.01. Meanwhile, after pollination with Moringa leaves, among the five groups showed no significant difference between the groups (p=0.488). Meanwhile, with One Way ANOVA test on the social interaction of the five groups after treatment showed no
significant difference between groups post-social interaction (p=0.488).

**Serum SOD Level**

Based on data on mean serum SOD level, the resulting data is not normally distributed and homogeneous (p=0.014; p=0.060). Data transformation using logarithm 10 will result in normal distributed data (p=0.830). One Way ANOVA test results showed a significant difference in serum SOD levels between groups (p<0.01).

**Table 2. Mean of Serum SOD Levels**

| Group           | Levels (U/ml)     |
|-----------------|-------------------|
| Negative Control| 174.77±11.94      |
| Positive Control| 108.97±3.775      |
| Treatment 1     | 117.42±3.107      |
| Treatment 2     | 125.32±2.042      |
| Treatment 3     | 137.04±5.41       |

SOD differences between groups can be determined by further tests are Tukey Post Hoc test shown in Table 2.

The ± results of Post Hoc Tukey test showed significant difference between K (-) with four other groups, between K (+) with P2 and P3, and between P1 to P3. These results also showed no significant difference between K (+) and P1, P1 and P2, and P2 and P3.

**Test memory**

Based on the homogeneity and normality test, the mean travel time in the modified memory test was homogeneous with p=0.299 (p>0.05) and was normally distributed with p=0.333 (p>0.05).

**Table 3. Mean of Memory Test**

| Groups           | Duration (second) |
|------------------|-------------------|
| Negative Control | 12.60±4.03        |
| Positive Control | 16.4±3.38         |
| Treatment 1      | 9.4±0.47          |
| Treatment 2      | 9.8±4.41          |
| Treatment 3      | 10±5.59           |

Although the graph shows the time difference between groups in reaching the platform, but from different tests using One Way ANOVA, p>0.05 (p=0.163), which means there is no significant difference in travel time in memory test between group.

**Level Zn**

One Way Anova test results showed p <0.01 which means there is a significant difference in the treatment group. Based on the result of post hoc Tukey obtained a significant difference between positive control group and negative control. However, there was no significant difference in the autism model group.

**Table 4. Average of Zn Levels**

| Groups            | Levels (mg/kg) |
|-------------------|----------------|
| Negative Control  | 1.07±0.27      |
| Positive Control  | 0.47±0.089     |
| Treatment 1       | 0.68±0.12      |
| Treatment 2       | 0.66±0.1       |
| Treatment 3       | 0.51±0.05      |

**Level Pb**

One Way Anova test results showed p <0.01. This means there is a significant difference in the treatment group. Based on the result of post hoc Tukey obtained a significant difference between positive control group and negative control. However, there is no significant difference in the autism model group.

**Table 5. Average Pb Level**

| Groups          | Levels mg/kg |
|-----------------|--------------|
| Negative Control| 0.0275±0.009 |
| Positive Control| 0.1725±0.002 |
| Treatment 1     | 0.215±0.05   |
| Treatment 2     | 0.15±0.03    |
| Treatment 3     | 0.21±0.04    |
Discussion

Feed intake
From the research that has been done before, it is known that the need to eat each day is 20-30 grams per day. Based on One Way ANOVA test result, $p>0.05$ ($p=0.27$). This means there is no significant difference in intake of feed between groups, so it is expected to change in rat treated during the study.

In rat fed Moringa oleifera powder with a dose of 180 mg, the edible oleifera leaves powder was 120.69 mg. Meanwhile, in rat fed oleifera leaves powder with a dose of 360 mg, oleifera leaves powder is eaten as much as 271.51 mg. Furthermore, in rat given oleifera leaves powder dose with a dose of 720 mg, oleifera leaves powder is consumed is as much as 454.97 mg.

Serum SOD level
The results of this study revealed significant differences between the Negative Control groups and the autism group. This could be because of the injection of NaVPA during pregnancy, so as to decrease serum SOD levels. In addition, Pb exposure in the sample may lead to a decrease in the level of SOD (Markiewicz-Górka et al., 2015). In this study showed elevated serum SOD levels in the autism group given oleifera leaves powder at a dose of 180 mg, 360 mg, and 720 mg. Large intake of Moringa leaves (Moringa oleifera) eaten in all three groups was 120.69 mg (67.05%), 271.51 mg (75.42%), and 454.97 mg (63.19%). These results suggest that elevated serum SOD levels are proportional to the given dose.

Flushing of oleifera leaves powder as an exogenous antioxidant may react with free radicals and increase endogenous SOD enzyme production to increase SOD levels. The antioxidant content in Moringa is very effective in preventing liver damage due to induction of hepatotoxin toxins. One of the antioxidant ingredients of Moringa aimed at binding to free radicals effectively is phenolic.

Memory Functions
The use of acetate in this study because exposure to acetate lead is easy to find in our environment and can also worsen the symptoms of autism in rat. As a result, the provision of lead acetate in the autism group rat model showed longer achievements to the platform on the Modified Morris Water Maze test, with an average time of 16.4 seconds compared to the normal group of rat with a time of 12.6 seconds. Although the achievement of travel time to the platform in the autism group rat model was longer than the normal group of rat, based on the results of different tests showed that there was no significant difference in short-term memory between autistic rat and normal rat. There was no difference in journey in achievement to the platform after four days of training time and the next day testing time between NaVPA-induced rats and NaVPA-induced rats (Markram et al., 2008).

Similarly, the NaVPA group induced and given moringa leaves powder, based on statistical analysis showed that there was no significant difference associated with the travel time to reach the platform compared to the group that was not given Moringa oleifera leaves powder. Nevertheless, groups of rat that were not given Moringa leaves powder had an average time to reach the platform longer than rat given leaves pine oleifera. Groups of rat not given moringa powder need an average of 16.4 seconds to reach the platform, whereas rats given a dose of 180 ml octopus leaves powder, 360 mg, 720 mg had an average time of reaching a platform of 9.4 seconds, 9.8 seconds, and 10 seconds. Compared with groups of rat not given leaves powder of oleifera, rat given oleifera leaves powder have better memory function by using modified Morris water labyrinth test. This is according to research conducted by Illiandri et al. (2010) that giving of leaves of Moringa varieties of...
NTB has a role in improving memory function in PEM rats because it contains antioxidant in oleifera. In this study, a group of rat with a dose of 180 mg of moringa leaves powder had the fastest time compared to rat fed moringa oleifera leaves powder 360 mg and 720 mg (Illiandri et al., 2010).

Moringa leaves have high amounts of vitamins and minerals that can function as natural antioxidants. Moringa leaves is a potential source of antioxidants (Illiandri et al., 2010). Various studies have shown that administration of antioxidants can prevent or treat a variety of toxic effects of lead, especially oxidative stress due to lead exposure. Generally, antioxidants can prevent lead toxicity in three ways, namely (1) disabling ROS at the molecular level so as to terminate the free radical chain reaction, (2) attract lead ions and prevent further ROS formation, and (3) attract lead and keep the situation redox, so that lead can not reduce oxygen molecules (Townsend, Tew, & Tapiero, 2003). The role of vitamins, especially vitamins B, C and E has been shown to combat significant toxicological manifestations of lead (Flora et al., 2012).

Zn Rat Model Autism by giving Moringa Leaves powder and Pb exposure.

Based on One Way Anova test results, it is known that there is a difference in Zn content. The post-hoc Tukey Test results show that there is a difference in Zn content in the K group with K +. Zinc levels in the blood in the K group (1.0725 mg/kg) were higher than the K + group (0.4675 mg/kg), this was due to dysfunction in autallothionein autistic child. This is characterized by severe Zn deficiency due to abnormalities of the glutationredox system (Yasuda, Kobayashi, Yasuda, & Tsutsui, 2013).

Based on the Tukey Post Hoc test showed that there was no difference in zinc levels in the K + group, P1 (with a dose of 180 mg of moringa leaves and consumed 60.34 mg), P2 (360 mg dose of moringa leaves and consumed 135.75 mg) and P3 moringa leaves 720 mg dose and consumed 227.5 mg). This condition occurs because the four groups come from the mother with the injection of NaVPA, so that Zn levels in these groups are not different.

In the K + group (0.4675 mg/kg) an increase in zinc levels in the treatment group 1 (0.675 mg/kg). Nutritional therapy using supplements that can stimulate metallothionein induction such as Zn, cysteine and glutathione, is able to restore metallothionein function of children's nutritional autistic disorder. Therapy by using supplements that can induce metallothionein induction such as Zn, cysteine and glutathione, can restore metallothionein function that is impaired in autism.

However in the main group in NaVPA the injection given the dander of multilevel Moringa leaves dose showed decreased levels of Zn. This is in line with the increasing dose given the powder of Moringa leaves. In a previous study, some factors that influence the absorption of zinc include other composition of nutrients contained in foodstuffs. Zinc absorption inhibitors include: fibers, phytates, oxalates, calcium, iron, and copper. Zinc competes with copper and iron absorption. Intervention between iron and zinc for absorption in rat suggests that there is antagonistic interaction (Bodiga & Krishnapillai, 2007).

At the molar ratio of Zn: Fe - 1:30, and Zn: Ca - 1: 667, significantly inhibits bioaccessibility Zn (Jayalakshmi & Platel, 2016). In 5 grams of Moringa leaves powder containing 0.12 mg Zn and 1.625 mg iron. Iron and calcium when administered at high doses significantly reduced status by inhibiting the absorption of Zn (Yang et al., 2014). Thus, in this study giving a dose of Moringa leaves powder storey of 180 mg, 360 mg and 720 mg per day containing Zn amounted to 0.0043 mg, 0.0086 mg and 0.0172
mg. In the dose of iron is increased as well, namely 0.058 mg, 0.117 mg, 0.234 mg. In this study a comparison between zinc and iron is 1: 14.5.

In other studies indicate that decreased concentration of zinc can reach 32% in serum when consumed be equal to calcium supplements (Jayalakshmi & Platel, 2016). In 5 grams of Moringa leaves contains 95 mg calcium (Witt, 2013) so in this study calculus contained in moringa leaves with multilevel dose is 3.42 mg, 6.84 mg and 13.68 mg. In this study a comparison between the zinc and calcium is 1: 855.

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Pb Rat Model Autism by giving the Oleifera Leaves Powder and exposure of Pb.

This study found an average difference between the level of Pb rat model of autism with normal rat. The normal rat had a mean Pb lower than the autism model rats equal to 0.0275 mg in the normal group of rat and 0.17 mg in the autistic rat model group. This is because autism has limitations in heavy metal excretion. The limitation of heavy metal excretion is associated with lower glutathione in autism (James et al., 2006) due to conjugation Glutathione is a pathway for removing heavy metals. The role of glutatation in lowering heavy metal levels through two routes is metal homeostasis and antioxidant protection. GSH Homeostasis through metal binds with heavy metals to reduce the amount of heavy metals freely in the blood. Meanwhile, through antioxidant protection GSH gives electrons to heavy metals so that heavy metals become stable, whereas GSH donated electrons will become reactive substances but GSH binds to others who have also donated electrons and formed GSSG. Reduction of GSSG is catalyzed by GSH reductase (GR) in a process requiring NADPH.

From the Tukey Post Hoc test showed that there was no significant difference between the groups that were given the powder of moringa leaves and the group that was not given the powder of Moringa leaves. However, there was an average decrease in lead levels in 2 treatment groups compared with the positive control group that was not given moringa leaves powder. This is because in 100 grams of moringa leaves contained 440 mg of calcium. According to another study calcium intake of less than 200 mg/day increases the incidence of lead poisoning (Bruening et al., 1997). In the treatment group 1 the amount of Moringa leaves powder consumed was 120.6 mg, and group 2 consumed 259.2 mg and treatment in group 3 was 460.8 mg, while calcium was needed to meet the demand of Moringa leaves powder by 197 mg.

The mean lead levels in the treatment groups 1 and 3 were higher than the positive control group. This is due to the influence of
protein contained in the powder of Moringa leaves. Moringa leaves powder contained in 24 grams/100 grams protein leaves powder. Based on research conducted Barltrop (1975) found that the administration of proteins with low doses or high doses will increase the absorption of Pb.

**Conclusion and Suggestion**

**Serum Levels of SOD**

Blood SOD levels in negative controls had the highest SOD levels and the lowest positive controls. SOD levels from positive controls (autism models without Moringa intervention) increased after being given an intervention and showed significant differences in significantly in the treatment group 2 and 3.

**Memory Function**

The administration of Moringa intervention (intervention group with Moringa powder) improves memory with a faster memory duration but still cannot improve significantly. Although there are differences in memory between the 5 statistical test groups, there has not been a significant difference.

**Levels of Pb and Zn**

There was a significant difference in the group of normal rat having the highest Zn levels and significantly different from the group of autism models who were either intervened with moringa or not (positive control). Intervention of Moringa leaf powder can increase Zn levels from autism without intervention but still not significantly.

As well as Pb levels, there was a significant difference between the Pb levels in the negative control group which had the lowest levels while the group with the intervention of Moringa leaf powder showed no significant difference to the positive control group.

**Social interaction**

The social interaction of positive control rats has the lowest social interaction.

Provision of kelor (intervention group with moringa powder) increases social interaction with lower duration of social interaction but still cannot improve significantly. Although there are differences in social interactions between the 5 groups, statistical tests have not shown significant differences.

**Suggestion**

Based on this study, further research is needed to increase the treatment group, ie normal rat and autism-exposed rat and time intervention.

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**References**

American Psychiatric, A. (2013). Diagnostic and statistical manual of mental disorders, Fifth Edition. https://doi.org/10.1176/appi.books.9780890425596.

Barltrop, D., Khoo, H. E. (1975). The Influence of Nutritional Factors on Lead Absorption. Postgraduate Medical Journal, 51(601), 795–800.

Birben, E., Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative Stress and Antioxidant Defense. WAO Journal, 5(1), 9–19. doi: 10.1097/WOX.0b013e3182439613.

Bjørklund, G. (2013). The role of zinc and copper in autism spectrum disorders. Acta Neuroboil Exp. (Wars), 73(2): 225-236.

Blumberg, S. J., Bramlett, M. D., Kogan, M. D., Schieve, L. a, Jones, J. R., & Lu, M. C. (2013). Changes in prevalence of parent-reported
autism spectrum disorder in school-aged U.S. children: 2007 to 2011–2012. National Health Statistics Reports, (65), 1–11.

Bodiga, S., & Krishnapillai, M. N. (2007). Concurrent repletion of iron and zinc reduces intestinal oxidative damage in iron- and zinc-deficient rats. World Journal of Gastroenterology, 13(43), 5707–5717.

Bruening, K., Kemp, F. W., Simone, N., Holding, Y., Louria, D. B., Bogden, J. D. (1999). Dietary Calcium Intakes of Urban Children at Risk of Lead Poisoning. Environmental Health Perspectives, 107(6), 431-435.

Chaste, P., & Leboyer, M. (2012). Autism risk factors: Genes, environment, and gene-environment interactions. Dialogues in Clinical Neuroscience, 14(3), 281–292. doi: 10.31887/DCNS.2012.14.3/pchaste.

Chen, Y. -W., Lin, H. -C., Ng, M. -C., Hsiao, Y. -H., Wang, C. -C., Gean, P. -W., & Chen, P. S. (2014). Activation of mGluR2/3 underlies the effects of N-acetylcysteine on amygdala-associated autism-like phenotypes in a valproate-induced rat model of autism. Frontiers in Behavioral Neuroscience, 8(June), 1–9. https://doi.org/10.3389/fnbeh.2014.00219

Christensen, D. L., Baio, J., Braun, K. V. N., Bilder, D., Charles, J., Constantino, J. N., … Yeargin-Allsopp, M. (2016). Prevalence and Characteristics of Autism Spectrum Disorder Among Children Aged 8 Years - Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. Morbidity and Mortality Weekly Report. Surveillance Summaries, 65(3), 1–23.

https://doi.org/10.15585/mmwr.ss6503a1

Company, C. C. (2010). Superoxide Dismutase Assay Kit, (706002), 1–5. https://www.caymanchem.com/product/706002/superoxide-dismutase-assay-kit.

Farr, S. A., Poon, H. F., Dogrukol-Ak, D., Drake, J., Banks, W. A., Eyerman, E., … Morley, J. E. (2003). The antioxidants α-lipoic acid and N-acetylcysteine reverse memory impairment and brain oxidative stress in aged SAMP8 mice. Journal of Neurochemistry, 84(5), 1173–1183. https://doi.org/10.1046/j.1471-4159.2003.01580.x.

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. Interdisciplinary Toxicology, 5(2), 47–58. https://doi.org/10.2478/v10102-012-0009-2.

Illiandri, O., Widjajanto, E., Mintaroem, K. (2010). Moringa oleifera Meningkatkan Fungsi Memori pada Tikus Model Kurang Energi Protein. Jurnal Kedokteran Brawijaya, 26(1), 28–31.

James, S. J., Melnyk, S., Jernigan S., Cleves, M. A., Halsted, C. H., Wong, D. H., Cutler, P., Bock, K., Boris, M., Bradstreet, J. J., Baker, S. M., Gaylor, D. (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am. J. Med. Genet B Neuropsychiatr. Genet., 141b(8), 947–956.

Jayalakshmi, S. & Platel, K. (2016). Compromised zinc status of experimental rats as a consequence of prolonged iron & calcium supplementation. Indian J. Med. Res., 143(2), 238–244.
La Fata, G., Weber, P., & Mohajeri, M. H. (2014). Effects of Vitamin E on Cognitive Performance during Ageing and in Alzheimer’s Disease. Nutrients, 6(12), 5453–5472. https://doi.org/10.3390/nu6125453.

Lidsky, T. I. & Schneider, J. S. (2005). Autism and autistic symptoms associated with childhood lead poisoning. The Journal of Applied Research, 5(1), 80–87.

Markiewicz-Górka, I., Januszewska, L., Michalak, A., Prokopowicz, A., Januszewska, E., Pawlas, N., & Pawlas, K. (2015). Effects of chronic exposure to lead, cadmium, and manganese mixtures on oxidative stress in rat liver and heart. Archives of Industrial Hygiene and Toxicology, 66(1), 51–62. https://doi.org/10.1515/aiht-2015-66-2515

Markram, K., Rinaldi, T., Mendola, D. L., Sandi, C., & Markram, H. (2008). Abnormal fear conditioning and amygdala processing in an animal model of autism. Neuropsychopharmacology, 33(4), 901–912. https://doi.org/10.1038/sj.npp.1301453

Mahardhika, R. V., and Budiono, I. (2012). Desain Interior Pusat Rehabilitasi Autisme dengan Konsep “Season in Wonderland Revolution Clinic” dan “ Free Running Building” sebagai Sarana Terapi Interior Partisipatif, Jurnal Sains dan Seni Pomits, 1(1), 1–6.

Rahayu, S. M. (2014). Deteksi dan intervensi dini pada anak autis. Jurnal Pendidikan Anak, 3(1) : 420-428.

Schultz, R. T. (2005). Developmental deficits in social perception in autism: The role of the amygdala and fusiform face area. International Journal of Developmental Neuroscience, 23(2–3 SPEC. ISS.), 125–141. https://doi.org/10.1016/j.ijdevneu.2004.12.012.

Townsend, D. M., Tew, K. D., & Tapiero, H. (2003). The importance of glutathione in human disease. Biomedicine and Pharmacotherapy, 57(3-4), 145–155. https://doi.org/10.1016/S0753-3322(03)00043-X

Wang, C. C., Lin, H. C., Chan, Y. H., Gean, P. W., Yang, Y. K., & Chen, P. S. (2013). 5-HT1A-receptor agonist modified amygdala activity and amygdala-Associated social behavior in a valproate-induced rat autism model. International Journal of Neuropsychopharmacology, 16(9), 2027–2039. https://doi.org/10.1017/S146114571300473

Witt, K. A. (2013). The Nutrient Content of Moringa oleifera Leaves. Echo, 1(1) : 1-6

Yang, G., Lai, C. S. W., Cichon, J., Ma, L., Li, W., Gan, W., -B. (2014). Sleep promotes branch-specific formation of dendritic spines after learning. Science, 344(6188), 1173–1178. DOI: 10.1126/science.1249098.

Yasuda, H., Kobayashi, M., Yasuda, Y., & Tsutsui, T. (2013). Estimation of autistic children by metallomics analysis. Scientific Reports, 3(1199): 1199. https://doi.org/10.1038/srep01199.