Comparison of NR2B Gene Expression Change in Wistar Rat Practicing Aerobic Exercise with Soybean, *Phyllanthus niruri*, and Combination of Soybean and *Phyllanthus niruri* Consumption

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Abstract

Objective: To examine the relationship between nutrition and brain memory.

Methods: This study was an experimental laboratory study conducted during the period of June 2011 to July 2012 at the Biomedical and Biochemistry laboratory, Faculty of Medicine, Universitas Padjadjaran. The subjects were 56 8-week-old male Wistar rats weighing approximately 200–250 grams which were divided into 8 groups with different treatments. The treatment groups received no exercise or exercise and soybean (*Glycine max), Phyllanthus niruri,* or combination of both.

Results: NR2B gene expression changes found is described as follows: (1) without practicing exercise (3.8) and after exercise (4.6); (2) *Glycine max* minus exercise (2.86) and *Glycine max* and exercise (3.17); (3) *Phyllanthus niruri* minus exercises (4.7) and *Phyllanthus niruri* and exercise (4.9); and (4) *Glycine max* and *Phyllanthus niruri* combination minus exercise (3.14) and *Glycine max* and *Phyllanthus niruri* combination and exercise (4.83).

Conclusions: This study determines that exercises and *Phyllanthus niruri* intake enhance NR2B gene expressions. *Glycine max* inhibits the NR2B gene expressions. *Glycine max* and *Phyllanthus niruri* combination, both with and without practicing exercises, enhance NR2B gene expressions. Therefore, practicing exercise and *Phyllanthus niruri* intake might cause brain cell apoptosis while *Glycine max* intake inhibits brain cell apoptosis.

Keywords: Exercise, gene expressions, *Glycin max*, NR2B, *Phyllanthus niruri*

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Introduction

Health and brain memory are the main factors and the most valuable assets influencing the development of a nation because they play important roles in creating qualified human resource. Some factors influence the attainment of a healthy and smart generation, including adequate nutrition and regular exercise. One of the widely consumed food that is considered rich in nutrition is soybean. Indonesians consume soybean in the forms of tofu, tempeh, bean sprout, and soybean milk. Soybean contains (1) nutrient including amino acid; (2) chemical compounds such as isovlavones, B vitamins (niacin, thiamine, and riboflavin), and C vitamin; and (3) mineral substances which include calcium, magnesium, calcium, phosphor, arginase enzyme, trigonelline, and other similar substances.[1] Shahib *et al*.[2] stated soy food should be consumed in an amount that is less than 5% of total calories per day. The consumption of soy food of 5–10% of the total calories per day is only allowed in a period of four weeks. Isoflavones in soy food as phytoestrogens modulate the estrogen level effectively and efficiently in human.[1]

Some studies have reported that soybeans enhance the spatial intelligence and prevent serious brain disorder in Wistar rat that had been given exercise using motor Morris Water Maze test (MWMT).[1,2] To measure the brain function changes at the molecular level, this

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study examined the NR2B gene expressions. There are several factors and circumstances which induce the NR2B gene expressions, such as soybean, glutathione, and exercise.[3] Other studies also reported that vigorous exercise may damage organs physiologically and, at the molecular level, induce apoptosis or even necrosis of several cells.[4,5]

Some studies reported that *Phyllanthus niruri* enhances the NR2B gene expressions and prevents glucotoxicity, thus inhibiting memory performance.[3,6] A wild tropical plant, *Phyllanthus niruri,* is well-known historically as traditional herb for generations.

The chemical compounds contained in *Phyllanthus niruri* are phenols: (-)-epicatechin, (+)-gallocatechin,(-)-epigallocatechin; flavonoids: kaempferol-4-rhamnopyranoside and eriodictyol-7-rhamnopyranoside, nirurin, nirurinetin, quersetin -3-O-glucopyranocide and rutin, astragalin, and fisetin-4'-O-glycoside; and steroids: doctriacontane acid, 24-iso-propyl-cholesterol and β-sitosterol.[1,3,6,7]

Exercise covers both physical activities and movements that stimulate physiological changes. Physiological adaptation of organ may result from regular exercises. As stated by the American College of Sport Medicine, exercise will improve functional capacity and quality of life, prevent risk factors associated with cardiovascular conditions, inhibit chronic diseases, improve psychological cognizance, and maintain social interactions.[4] Exercising also inhibits several chronic diseases, such as Alzheimer syndrome.[8] It is recommended to exercise 30 minutes per day, 4 times a week, to improve muscle strength, coordination, and stabilization as well as gaining body health.[4] One of the popular weigh-bearing exercises is aerobic, which is a distinguishing exercise to prevent degenerative diseases.[5]

Brain memory is a functional cognitive capability to retain information and knowledge. It is capable to keep, maintain, and reorganize information. Some cognitive psychology and surgery studies reported that memory is an individual's ability to record sensory stimulus and to recall past events in any circumstance. One of the several genes which may stimulate brains is NR2B.[9,10]

This study was initiated to examine the influence of exercise and food intake (*Glycine max* and *Phyllanthus niruri*) in inducing the brain (brain memory). The NR2B gene expressions were used as the indicator to measure brain change in Wistar rats before and after exercising and *Glycine max* and *Phyllanthus niruri* intakes. In addition, some anatomy pathology tests were performed to examine apoptosis and new synapses.

Methods

This study applied the quantitative approach and an experimental laboratory method with natural formula product oral administration. The study was conducted in the period of June 2011 to July 2012 at the Biomedical and Biochemistry laboratory, Faculty of Medicine, Universitas Padjadjaran. The subjects of this study were 8 week male Wistar rats, weighing approximately 200–250 grams. The subjects were subjected to low intensity exercise using treadmill with average 50–69% VO_2 max, 18–20 m/minute in 30 minutes for 14 days. The exercise was a free variable while the NR2B gene expressions were the bound variable.

The examination involved 56 male Wistar rats (Federer co-area formula) which were divided into 8 groups: Group A: control group; Group B: did not exercise; Group C: exercised and received less than 5% *Glycine max* infusion intake; Group D: did not exercise and received less than 5% *Glycine max* infusion intake; Group E: exercised and received *Phyllanthus niruri* infusion intake; Group F: did not practice exercise and received *Phyllanthus niruri* infusion intake; Group G: exercised and received *Glycine max* and *Phyllanthus niruri* infusion intake, Group H: did not exercise and received *Glycine max* and *Phyllanthus niruri* infusion intake.

Table 1 NR2B Gene Expressions in Negative and Positive Control Groups

No.	Groups (Gene Expression)		Average Difference (%)
	Negative Control	Positive Control (Practicing exercise)	
1	5	5	
2	4	5	
3	4	5	
4	3	5	
5	4	4	
6	3	3	
7	-	5	
Average	3.8	4.6	21.05

Glycine max and *Phyllanthus niruri* were obtained from Sumedang, West Java, Indonesia. They were then processed into infusions, both individually and in combination. All Wistar rat NR2B gene expressions of the samples and control were examined using RT-PCR method.[12,13] Electrophoresis results were then analyzed using the densitometry Scion Image program by comparing to GAPDH genes.

Results

The NR2B gene expression assay was used to evaluate the changes in Wistar rat brain function after exercise and *Glycine max* and *Phyllanthus niruri* intakes. To examine related gene expressions, the NR2B gene expressions were determined before practicing exercises and respite period (Table 1). The gene expressions were tested using RT-PCR and the results were 3.8.

The NR2B gene expression assay on respite period showed various results, from weak to moderate. The average of gene expressions increased to 21% (4.6) on average after the exercise. No statistical significant differences (>0.05) were found; thus, exercises enhanced the NR2B gene expressions from weak to moderate. This condition might cause serious impact to brain functions.

The superficial NR2B gene expressions have to be controlled since the high level of the NR2B gene expressions will damage the brain function. In addition, exercise effectively enhances the NR2B gene expressions.

Glycine max intake enhanced the NR2B gene expressions when compared to the intake of *Glycine max* and exercise (Table 2). Both *Glycine max* intake and lack of exercise inhibited the NR2B gene expressions when compared to control (without exercise). The inhibition point (20.6%) was normal; thus, exercise enhanced NR2B gene expressions moderately. Therefore, *Glycine max* intake and exercise enhanced NR2B gene expressions.

Phyllanthus niruri intake enhanced NR2B gene expressions significantly although the rats did not exercise (Table 3). *Phyllanthus niruri* intake and exercise enhanced the NR2B gene expressions, which induced intelligent and memory.

Apoptosis may be induced by inappropriate high level NR2B gene expressions; previous studies showed that ≥40% of total calorie dosage causes neurotoxin in rats.[11–13]

Some studies describe that low dosage of *Glycine max* supplement enhances rat NR2B gene expressions and memory.[3] *Glycine max* and *Phyllanthus niruri* combination was shown as enhancing high level of NR2B gene expressions (Table 4).

Table 2 NR2B Gene Expressions in *Glycine max* Intake without Exercise Group Compared to *Glycine max* Intake and Exercise Group

No.	Groups (Gene Expression)		Average Difference (%)
	Glycine max intake without Exercise	*Glycine max* Intake and Exercise	
1	2	3	
2	3	3	
3	3	4	
4	4	4	
5	3	3	
6	3	2	
7	2	-	
Average	2.86	3.167	21.06

Table 3 NR2B Gene Expressions in *Phyllanthus niruri* Intake without Exercise Group when Compared to *Phyllanthus niruri* Intake and Exercise Group

No.	Groups (Gene Expression)		Average Difference (%)
	P. niruri Intake without Exercise	*P. niruri* Intake and Exercise	
1	5	5	
2	5	5	
3	3	5	
4	5	5	
5	5	5	
6	5	5	
7	5	4	
Average	4.7	4.9	4.26

Table 4 NR2B Gene Expressions in *Glycine max* and *Phyllanthus niruri* Composition Intake without Exercise Group Compared to *Glycine max* and *Phyllanthus niruri* Composition Intake and Exercise Group

No.	Groups (Gene Expression Score)	
	***Glycine max* and *Phyllanthus niruri* Combination Intake without Exercise**	***Glycine max* and *Phyllanthus niruri* Combination Intake and Exercise**
1	2	4
2	3	5
3	4	5
4	4	5
5	5	5
6	2	5
7	2	-
Average	3.14	4.83

The treatment and exercise enhanced more than 50% of NR2B gene expressions. Furthermore, *Glycine max* and *Phyllanthus niruri* combination intake and exercise were effective to enhance NR2B gene expressions when compared to *Glycine max* intake only.

Phyllanthus niruri intake minus exercise enhanced gene expressions when compared to *Phyllanthus niruri* intake and exercise, which resulted in slight differences in gene expressions ($p>0.05$).

The gene expressions increased after receiving *Phyllanthus niruri* and exercising compred to the respite period. *Phyllanthus niruri* intake without practicing exercise was 4.7 while exercise without *Phyllanthus niruri* intake was 4.6. The NR2B gene expressions were moderate. These results described that *Phyllanthus niruri* intake and exercise enhanced the gene expression at the same level.

Glycine max intake or exercise inhibited the NR2B gene expressions (less than 26%). The combination of *Glycine max* intake and exercise inhibited the NR2B gene expressions of approximately 30.4%. In contrast, *Phyllanthus niruri* intake and exercise enhanced the NR2B gene expressions of approximately 23%.

It is apparent that *Glycine max* has the opposite side effects to *Phyllanthus niruri*. *Glycine max* inhibition effect was tested in rats which were also given *Phyllanthus niruri* without exercise. *Glycine max* and *Phyllanthus niruri* combination intake and exercise enhanced the NR2B gene expression (4.83). *Glycine max* tended to have inhibiting effects towards brain cell apoptosis.

Discussion

This study determines that exercise enhances the NR2B gene expressions in Wistar rats.[14] The NR2B gene expressions of rats that received *Glycine max* intake and did not exercise were lower than those that received *Glycine max* intake and exercised. *Glycine max* inhibited the NR2B gene expressions in groups both with or without exercise.

The NR2B gene expressions of rats that received *Phyllanthus niruri* intake and did not exercise were lower than those that received *Phyllanthus niruri* intake and exercised. *Phyllanthus niruri* without exercise enhanced the NR2B gene expressions. *Phyllanthus niruri* and exercise slightly enhanced the NR2B gene expressions.

The NR2B gene expressions of rats that received *Glycine max* and *Phyllanthus niruri* combination and did not exercise were lower than those that received *Glycine max* and *Phyllanthus niruri* combination and exercised. *Glycine max* and *Phyllanthus niruri* combination intake without exercise inhibites the NR2B gene expressions. In contrast, *Glycine max* and *Phyllanthus niruri* combination and exercise enhanced the NR2B gene expressions.

The NR2B gene expressions are enhanced after exercising. The NR2B gene expressions of rats that received *Glycine max* intake are also enhanced. *Glycine max* intake and exercise inhibited NR2B gene expressions.

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