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TEMPE, TOFU AND AMYLOID BETA 1-40 SERUM LEVELS IN OVARECTOMIZED RATS

Atik Kridawati^{1,3*} Ahmad Sulaeman², Tri Budi W. Rahardjo³, Eef Hogervorst⁴

¹Public Health Graduate Program, University of Respati Indonesia

²Community Nutrition Department, Faculty of Human Ecology, Bogor Agricultural Institute

³Centre for Family and Ageing Studies, University of Respati Indonesia

⁴School of Sport, Exercise and Health Sciences, Loughborough University, UK

*Correspondence: Atik Kridawati

Address: University of Respati Indonesia. Jalan Bambu Apus I No.3 Cipayung, Jakarta Timur
13890. Indonesia

Email: atik @urindo.ac.id

Abstract

Estrogens have been found to reduce beta amyloid levels, a risk factor associated with dementia. We hypothesized that phytoestrogenic soybean products such as tempe and tofu might show similar effects. The aims of this study were to analyze the effect of tempe and tofu flour on beta amyloid1-40 serum levels in elderly rats. This research was conducted on female Sprague Dawley rats, aged 12 months. Before the intervention rats underwent ovariectomy (OVx) and were grouped into 5 intervention groups which were given either: tempe flour, tofu flour, estradiol, and casein as an active control. There was also a non OVx control group which was fed a normal diet. The intake of tempe and tofu flour decreased beta amyloid serum levels in all estrogen and phytoestrogenic treatment groups, offsetting effects of OVx (but not in casein group, where beta amyloid levels rose). However, the tempe flour group showed the strongest decrease in serum beta amyloid levels compared to the other groups. Future studies should investigate whether tempe can reduce beta amyloid levels in patients with dementia.

Keywords: tempe flour, tofu flour, beta amyloid, ovariectomized rats

Background

Alzheimer's disease (AD) is a progressive neurodegenerative disease, which is the main cause of dementia in elderly subjects (Alvarado et al. 2016). One of the main characteristics of AD is loss of memory. Beta amyloid peptide levels are also elevated in AD brain and blood and precede the cognitive impairment (Wang *et al.* 2006, Smith and Grenebreg, 2009). The prevalence of AD is around 1.5% at age 65 years, doubling every 4 year to reach ~30% at age 80 years. The risk of AD is also higher in older women than in men, which may be related to their lower levels of sex steroids, such as estrogens after menopause (Hogervorst, 2000; Mazure and Swendsen, 2016). Estrogen depletion and

surgical menopause at an earlier age further increase dementia risk (Rocca, 2007, Hogervorst, 2010, Bhatta, 2018). Other risk factors which are associated with cognitive impairment and dementia are genetic factors, but also treatable factors associated with cardiovascular disease, such as diabetes mellitus, depression, hypertension, engaging in less physical and social activities, smoking, high total cholesterol, and obesity (Kivipletyo, 2005; Whitmer et al, 2005; Oliveira, 2017). Lastly, low folate and cobalamin (vitamin B12) levels intake increase risk for dementia (Smith, 2008, Setyowati et al, 2019, Nurniawati, 2015; Kidawati et al, 2015, Hogervorst et al, 2017).

Estrogen can have effects on many organ systems, including the brain (Gruber 2002; Hogervorst, 2010, Hogervorst 2011) through the presence of the estrogen receptor (Kenneth *et al.* 1999). Estrogen Replacement Therapy (ERT) may have beneficial effects on ageing process, but its use in older women could confer risk. ERT was shown to protect against the development of AD (perhaps by acting on beta-amyloid) according to meta-analyses of observational trials, but treatment study results indicate that an older age and longer duration of treatment (> 1 year) may actually confer risk for dementia and restrict its use to recently menopausal women for short periods of time (Hogervorst, 2007;2010). Prolonged use of estrogens in older women may confer risk for dementia possibly through its effect on promoting apoptosis in neuronal cells undergoing pathological change, which is more likely in older women (Hogervorst 2012, Brinton-Diaz, 2008).

Phytoestrogens are a group of biologically active plant substances with a chemical structure that is similar to that of estradiol, the most potent endogenous estrogen (Bhanthena 2002). The three major classes of phytoestrogens are isoflavones, lignans, and comestans and the major bioactive isoflavones are genistein and daidzein, as well as equol (which is produced in the intestines) (Bhanthena 2002). Isoflavones may have positive effects on cognitive function and may also reduce beta amyloid (Pan 2000; Caroline 2010, Alireza 2008). Isoflavone sources are soybean and soybean products. Indonesia is a country where people commonly consume large amounts of tempe and tofu, soy products rich in phytoestrogens. Tempe consumption was shown to also increase serum estrogen (Kridawati, 2019).

In a previous human observational study, high tofu consumption was associated with worse memory, although high tempe consumption (a fermented whole soybean product) was independently related to better memory, particularly in Indonesian participants over 68 years of age (Hogervorst *et al.* 2008). Similarly, data from the Honolulu-Asia Aging Study (HAAS) in Japanese Americans also showed that higher midlife tofu consumption was

independently associated with indicators of cognitive impairment and brain atrophy in late life (White 2000). Age may thus modify the effect of phytoestrogens on cognition, similar to the effect of estrogens (Hogervorst, 2010, 2009, 2014; Kridawati 2013, 2016; Soni et al, 2016). In middle-aged Indonesian women optimal levels of genistein were associated with better memory (Hogervorst 2008). The positive effect of tempe regardless of age may be explained by its soybean fermentation which produces folate and cobalamin. Folate can reduce the negative association of high estrogen with adverse brain function in older women (Hogervorst, 2003, 2017, Setyowati et al, 2019). We hypothesized that because phytoestrogens have half the bioactive effect of estrogens (due to their lower binding capacity to the estrogen receptor or ER) that effects on beta amyloid would be strongest in OVX rats given estradiol, followed by tempe and tofu flour.

Method

Design

The study was conducted using an experimental design with pre-post-treatment controls at the animal hospital and Histology Laboratory of the Faculty of Veterinary Medicine, Bogor Agricultural University. Spargue Dawley strain female rats aged 1 year (n=72) were obtained from the Faculty of Veterinary Medicine, Bogor Agricultural University. Rats were placed in a plastic cage with a lid made of wire covered with chaff ram, and given drinking water ad libitum. The environment was not humid with adequate ventilation and adequate lighting with cycles of 14-hour light and 10 hours of darkness.

Rats were fed modified flour given three months after ovariectomy surgery. Feed before the intervention was derived from PT. Comfeed Indonesia. Composition of nutrients in flour was: 18-20% crude protein, 40% crude fat, 7% crude fiber, 2% calcium and phosphorus, 13% ash and 10% water. Rats were then grouped into 5 groups: tempe flour, tofu flour, estradiol, casein after ovariectomy (OVx), and non ovariectomized controls given normal feed without additions. Three months after OVx, diets were started. Dietary interventions were carried out for 2 months using the different feed compositions according to the AIN-93m which consisted of 14% protein, 5% minerals, 4% fat, 1% vitamins, 5% fibre and 66% starch. The active control group was given casein as a protein, the passive control group was also given casein but had not undergone ovariectomy. Tempe and tofu were substituted for casein as active treatments. Another group was given casein and estradiol 3 months after ovariectomy. In the estradiol group treatment consisted of ethinylestradiol

(synthetic estrogen) of 9×10^{-3} mg / day/200g B by using a sonde. Nutrient content of the tempe and tofu flour was assessed using HPLC in the laboratory in Bogor.

Point observations were made at a time before ovariectomy called baseline 1 (BS₁), three months post-ovariectomy called baseline 2 (BS₂), two weeks after the intervention (I₂), five weeks after the intervention (I₅), and eight weeks after the intervention (I₈).

Beta amyloid in serum assays were carried out at the Reproduction and Rehabilitation Unit (URR) Laboratory in the Faculty of Veterinary Medicine, IPB. Analysis of beta amyloid in serum was done using the ELISA method with Amyloid Beta Peptide 1-40 (Ab1-40)(E90864Ra) reagent.

Processing and Data Analysis

Data obtained were processed and analysed statistically to investigate differences between the treatment groups using analysis of variance (ANOVA) repeated measures with post hoc Tukey tests and polynomial contrasts in SPSS 17.0

RESULTS

Intake of tempe flour (4,4 gram/day) was equivalent to intake of folic acid of $1,104 \times 10^{-3}$ ppm, vitamin B12 of 4.27×10^{-2} mcg and 22.25 mg isoflavones, while tofu flour only contained 10.16 mcg isoflavones and no B vitamins. Results of this study (fig 1) showed that rats had a significant increase in serum beta amyloid three months after ovariectomy. The tempe (fermented soybean) flour intervention group from the second week after until the fifth week of treatment showed a significant decline in beta amyloid levels in serum which dropped below that of controls. After eight weeks beta amyloid levels began rising slightly again, although these remained significantly lower compared to casein treatment using repeated measures ANOVA post hoc Tukey tests ($p < 0.05$). Tofu flour also induced a decline in beta amyloid levels after two weeks of treatment until after eight weeks of intervention. However, the beta amyloid level in serum remained higher than that of the rats consuming tempe flour and seemed to only offset the effect of OVx. The estradiol group intervention had similarly decreased beta amyloid in serum offsetting effects of OVx on beta amyloid. In the casein group beta amyloid levels increased at two, five and eight weeks after intervention over that of all (phyto)oestrogenic compounds. This was in contrast to the non ovariectomized group, where the level of beta amyloid tended to remain the same.

Table 1. Mean $\pm$ SD (CI) and p values of Amyloid Beta in Serum

BS ₁	119,22± 20,27 (86,97-151,48)					
	OVx				Non OVx	P value
BS ₂	172,4±30,71 (123,52-221,27)				114,22±33,95 (60,19-168,25)	0,998
	Intervention groups					
	Tempe Flour	Tofu Flour	Estradiol	Casein	Non Ovariectomy	P value
I ₂	85,9±16,4 (59,79-112,0)	105,57±35,24 (49,49-161,65)	102,52± 20,39 (70,09-134,96)	129±28,03 (84,89-174,11)	109,85±37,88 (49,57-170,12)	0,682
I ₅	76,52± 34,14 (22,19-130,85)	104± 24,26 (65,67-142,88)	108,52± 28,27 (63,54-153,51)	163,82± 95,8 (11,39-316,26)	102,77± 5,07 (94,69-110,85)	0,033
I ₈	93,37± 22,31 (57,88-128,87)	103,82± 24,32 (65,12-142,53)	111,1± 28,93 (65,06-157,14)	142,35± 34,07 (88,14-196,56)	101,7±4,96 (93,81-109,59)	0,293

Figure 1. Beta-amyloid levels in serum across different treated groups over time**Table 2. Mean $\pm$ SD (95% CI) difference in beta amyloid (pg / ml) for time of intervention with baseline 2**

	2 weeks - <i>baseline 2</i>	5 weeks - <i>baseline 2</i>	8 weeks - <i>baseline 2</i>
Tempe Flour	-33,32 $\pm$ 19,87 (-75,68-9,03)	-23,12 $\pm$ 19,87 (-19,23-65,48)	-42,7 $\pm$ 19,87 ^a (-85,06-(-0,34)
Tofu Flour	-13,65 $\pm$ 19,43 (-55,09-27,77)	-14,95 $\pm$ 19,43 (-56,37-26,47)	-15 $\pm$ 19,43 (-56,82-26,02)
Estradiol	-10,27 $\pm$ 34,25 (-62-83,29)	-44,6 $\pm$ 34,25 (-28,41-117,61)	-16,7 $\pm$ 34,25 (-89,71-56,31)
Casein	-5,0 $\pm$ 20,35 (-48,38-38,38)	-10,7 $\pm$ 20,35 (-54,08-32,68)	-8,12 $\pm$ 20,35 (-51,51-35,26)
Non	-16,47 $\pm$ 17,11	-9,4 $\pm$ 17,11	-8,32 $\pm$ 17,11
Ovariectomy	(-19,96-52,94)	(-27,06-45,86)	(-26,14-44,79)

Note: a=P value=0.048.

The tempe flour group had the largest change in beta amyloid levels after eighth weeks of intervention ($P < 0.05$). The lowest beta amyloid content was found in the tempe flour group, which was below baseline. The difference in beta amyloid levels in the tofu, estradiol, and casein flour groups from baseline 2 was not significant (Table 2).

DISCUSSION

Estrogen reduces the risk of Alzheimer's disease (AD) in postmenopausal women, beta-amyloid levels ($A\beta$) in animal AD models, and $A\beta$ secretion from neuronal tissue (Jeffrey et al. 2002). Giving ERT can improve cognition and delay development of AD. The exact biological mechanism of estrogen as a *neuroprotective* agent is unknown. ERT can slow the occurrence of AD by reducing the release of $A\beta$, the main component of the amyloid plaque, to the brain parenchyma (Brinton 2008). This phenomenon was first described in non-neuronal and neuronal tissue cells. In animal models, guinea pigs and transgenic mice show that postoperative estrogen administration can decrease $A\beta$ levels. There was an inverse relation between 17β -E₂ and $A\beta$ 42 in cerebrospinal fluid in female patients with AD (Schonknecht et al. 2001).

Estrogen mechanisms that regulate beta amyloid have not been fully explained, although this sex hormone is involved in both regulating the production and clearance of beta amyloid (Gruber 2002). In this study in serum, estrogen levels dropped significantly after ovariectomy which was followed by an increase in serum levels of $A\beta$ 1-40. The increase in the lag time cannot be explained by considering $A\beta$ 40 clearance from the brain which occurs in a short time (a few minutes) (Zhang 2011). Beta amyloid is the result of the production of proteolytic cleavage of its parent protein, the amyloid precursor protein (APP). The majority of APP is metabolized by two pathways. In the amyloidogenic pathway, APP is cleaved by beta-secretase and gamma secretase, liberating the $A\beta$ peptide largely from the chain of amino acids 40 and 42. In the non-amyloidogenic pathway, APP is cleaved in the β A domain by alpha secretase, preventing the formation of the full-length $A\beta$ peptide, but releasing a solution in the form of a protective APP called alpha APP. Cell culture studies showed that estradiol and testosterone can improve the processing of APP by promoting the non-amyloidogenic pathway, thereby reducing the production of $A\beta$ (Jaffe 1994, Zhang 2011).

Serum beta amyloid levels in the ovariectomy group showed a significant increase from baseline 1. Increased $A\beta$, due to ovariectomy, could be caused a loss of negative

estrogen feedback to gonadotropin *lutein hormone* (LH), thereby causing an increase in LH levels which *in vitro* was also proven to increase beta amyloid production through the amyloidogenic pathway (Brinton, 2008). Giving tempe flour to mice showed a decrease in serum A β . At eighth weeks of intervention, the tofu flour group had a serum beta amyloid content 1.11 times lower than the tofu flour group. Compared with the estradiol and casein groups the tempe flour group A β results were 1.2 and 1.5 times lower, respectively. Beta amyloid in the tofu flour group was almost the same as non-ovariectomy group showing off-setting of OVx similar to the estrogen treatment.

Vitamin B12 (cobalamin) and folate are important cofactors in several biochemical reactions, including the conversion of homosysteine to methionine and the synthesis of SAME (S-adenosylmethionine). These reactions are believed to be crucial in maintaining neurological health (Miller, 2002). While observational studies in humans did not show an association between these B vitamins and serum beta amyloid 1-40 or 1-42 levels (Gu 2012), another study showed that a diet deficient of B vitamins induced increased Abeta levels and depositions in hippocampus and cortex in a transgenic mouse model of AD-like amyloidosis (Zhuo 2010). Another rat study also showed that hyperhomocysteinemia (induced by these B vitamin deficiencies) increased Abeta production through the enhanced expression of gamma-secretase and APP phosphorylation, causing memory deficits that could be reversed by folate and vitamin-B12 treatment. The authors suggested that hyperhomocysteinemia may serve as an upstream factor for increased Abeta production as seen in patients with Alzheimer disease (Zhang, 2009).

In this study we showed that tempe flour has the potential to reduce beta amyloid in ovariectomised rats below baseline levels. Further research needs to be done to investigate the effect of tempe flour consumption in older female rats (3 years old), and male middle-age and older rats. Considering its safety profile, tempe treatment studies should commence in female patients who are planned to undergo OVx and in those who are afflicted with AD.

CONCLUSION

A regular high intake of phytoestrogens derived from tempe flour may have positive effects on brain function by reducing beta amyloid levels in elderly female rats.

FUTURE SUGGESTIONS

Future studies should include younger and older male rats and further identify beta amyloid mechanisms related to tempe consumption.

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