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The effect of extract purple sweet drop on nuclear factor kappa B and amyloid deposits in D-galactose induced dementia in rats

Ketut Widyastuti, Anak Agung Ayu Putri Laksmidewi, I Made Oka Adnyana

Deparment of Neurology, Udayana University/Sanglah Hospital, Denpasar, Bali, Indonesia

ABSTRACT

Background. Alzheimer's dementia (AD) as a neurodegenerative disease requires preventive measures to reduce its progression. Beta amyloid plaques induce chronic inflammation and apoptosis in AD. Anthocyanins in purple sweet potato are potential for prevention and therapy of AD.

Aim. This study aimed to determine the role of anthocyanins in purple sweet potato extract to prevent inflammation so as to provide a neuroprotective effect on D-galactose-induced rats.

Material and methods. We used a randomized posttest only control group design. 32 male Wistar rats according to eligibility criteria were randomized into control and treatment groups. The treatment group was given purple sweet potato extract at a dose of 200mg/kgBW every day for 70 days. Both groups on day 15 induced dementia using D-galactose. Anti-inflammatory activity was evaluated from the examination of NFkB levels by ELISA technique and neuroprotective effects by immunohistochemical examination of amyloid plaque deposits.

Results. The mean NFkB of the treatment group (1417.55+255.82) was lower than the control group (1672.23+202.80) which was significant ($p<0.05$) and the amyloid plaque deposits in the treatment group were thinner than the control group. control.

Conclusions. Administration of purple sweet potato extract to D-galactose-induced rats caused lower NFkB levels and thinner amyloid plaque deposits. The research implication is that administration of purple sweet potato extract can prevent inflammation and provide neuroprotection effects in D-galactose-induced rats.

Keywords: anthocyanins, amyloid plaque, NFkB, purple sweet potato

INTRODUCTION

Dementia is a neurodegenerative disease in the form of a syndrome of progressive decline in intellectual abilities in the elderly that causes cognitive, behavioral and daily functional activities. Dementia will cause dependence on the elderly (elderly) in their activities so that it becomes a burden for families, communities and the government. Efforts are needed to maintain cognitive abilities so that the quality of life of the elderly remains good. The most common cause of dementia is Alzheimer's dementia (DA), which in the early phase is characterized by a progressive decline in memory function and then develops involving several cognitive domains and behavioral changes (1).

The main neuropathological signs in AD are extracellular amyloid beta (Aβ) plaque deposition, neurofibrillary tangle (NFT) formation, chronic neuroinflammation and loss of cholinergic neurons. Chronic inflammation is the main mechanism that induces its occurrence. Alzheimer's dementia (AD). The neuroinflammatory process of AD is triggered by chronic deposition of amyloid protein (Aβ) by activating microglia as immunocompetent cells in the brain to release proinflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6) and

Corresponding author:
Ketut Widyastuti
E-mail: kt_widyastuti@unud.ac.id

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tumor necrosis factor-α (TNF-α) (2). Activation of microglia has a dual effect on the development of AD which can reduce Aβ accumulation by increasing phagocytosis, cleansing and degradation thus preventing the formation of amyloid plaques in the brain but on the other prolonged activation of microglia leads to the release of pro-inflammatory cytokines and contributes to neuronal damage (3).

Aβ deposits activate the acute immune response of microglial cells and astrocytes. Simultaneously Aβ plaques also produce and activate several inflammation-related proteins such as complement factors, acute phase proteins, chemokines and cytokines (2). The role of TNF- signaling has also been reported in abnormal amyloid precursor protein (APP), accumulation of Aβ plaque and pathological tau protein, and cell death. Increased levels of TNF- in the brain can inhibit Aβ clearance in microglia, cause synaptic dysfunction and cause widespread neuronal death, accelerating cognitive decline (4). Signaling via cognate TNF-R receptors elicits distinct cellular responses in terms of cell proliferation, cell migration, and apoptosis mediated through activation of several signal transduction cascades involving nuclear factor kappa B (NF-κB), c-Jun N-terminal kinases (JNKs), p38, role of sphingomyelinase and ceramide (3).

Purple sweet potatoes have been studied in several countries and are known to contain high levels of flavonoids, especially anthocyanins. Anthocyanin as a natural antioxidant in purple sweet potato (*Ipomoea batatas* L) from Bali cultivar has an antioxidant effect by suppressing the production of malondialdehyde (MDA) in vivo and inducing endogenous antioxidants (5). Anthocyanins have unique antioxidant properties because they can destroy reactive oxygen species (ROS) and reactive nitrogen species (RNS) directly which is judged by their high oxygen radical absorption capacity and increased intrinsic antioxidant defense of cells (6).

Rodents have been widely used in AD research because of their relatively large similarities in physical structure and cognitive systems, as well as their availability and relatively low cost compared to primate systems. The D-galactose model can be used for the study of aging and aging-related neurological disorders including AD (7). Administration of excessive doses of exogenous D-galactose beyond normal concentrations can induce aging effects in several organs by increasing the formation of ROS that cause mitochondrial dysfunction, oxidative stress, inflammation and apoptosis in nerve cells (8). In dementia model mice, a gradual increase in the number of Aβ plaques has been reported (9). A pre-elimination study on 20 Wistar rats given d-galactose at a dose of 100 mg/KgBW for 8 weeks showed MDA levels and mean of spatial memory scores in the intraperitoneal injection group were a little than oral group with $p < 0.05$ (10).

The anthocyanin-rich extract significantly reduced the production and secretion of nitric oxide, IL-1β and TNF-α. The modulation of the proinflammatory signaling pathway by anthocyanins was assessed by the significantly decreased levels of c-JNK, p38-MAPK, ERK1/2 and Akt activation. Reduced activity of this signaling pathway correlates with reduced activation of nuclear factor-κB (NF-B) and prevents its translocation to the nucleus, which may mediate transcription of pro-inflammatory genes (11). Several studies have shown that anthocyanins are potent inhibitors of NFkB. The presence of NFkB inhibition prevents chronic inflammation and increases brain plasticity, which is characterized by thinning of amyloid plaques. This study is expected to be a further study on the benefits of anthocyanins in the management of dementia and NF-κB as an appropriate marker in assessing these changes.

MATERIAL AND METHODS

This study used an experimental design with a posttest control group design to compare an aqueous extract of purple sweet potato given to rat models of dementia with D-galactose induction, resulting in lower NF-kB levels than controls and resulting in thinner amyloid plaque deposits than controls. This research was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Udayana University for 6 months from February to July 2021.

This research has received approval from the research ethics commission of the Faculty of Medicine, Udayana University / Sanglah Central General Hospital Denpasar with No. 1032/UN 14.2.2VII.14/LT/2020

The sample population in this study was 36 samples of male Wistar rats aged 12-14 weeks weighing 200-300 g kept in the Pharmacology Laboratory of the Faculty of Medicine, Udayana University which were divided into 2 groups, control and treatment with exclusion criteria of sick and hyperactive rats.

Descriptive data analysis of spatial memory disorders to see a description of the basic characteristics of the subject based on the research group. Data on levels of NF-kB and amyloid plaque deposits were expressed on a numerical scale, normality test was performed using the Shapiro Wilk test and if the data were normally distributed then an independent parametric t test was performed and if the data were not normally distributed, the Mann Whitney non-parametric test was used to find the difference between the two. group. The level of significance with p, and 95% confidence interval. However, if the drop out is more than 15%, an intention to treat analysis will be carried out. All data obtained from this study were analyzed using the SPSS 20.0 for Windows program.

RESULTS

The initial subjects in this study were 36 rats divided into 2 groups, namely the control group and the treatment group, each of which consisted of 18 rats. However, 2 mice from each group dropped out because the mice experienced death and tissue damage occurred during sample delivery. The number of samples for each group became 16 rats and this number already met the minimum sample size criteria for research.

Research on male wistar rats aged 12-14 weeks with a body weight of 200-300gram was carried out for 70 days. The control group received D-galactose and aquabidest while the treatment group received D-galactose and anthocyanin. Induction of d-galactose at a dose of 100 mg/kg/day via intraperitoneal injection was performed once a day at 08.00 am. The administration of purple sweet potato extract at a dose of 200 mg/kgBW/day orally was administered via a nasogastric tube once a day at 08.00 am. After 70 days of treatment, the rat brain tissue was taken for examination of NFkB by ELISA technique and examination of amyloid plaque deposits by immunohistochemical technique.

The results of the examination of NFkB levels in the treatment group averaged 1417.55+255.82pg/mg while in the control group it was 1672.24+202.80pg/mg. The results of the independent t test analysis in Table 1 showed that the purple sweet potato water extract caused the mean NFkB levels in the treatment group to be significantly lower than the control group after D-galactose induction ($p < 0.05$).

In the immunohistochemical examination, amyloid plaque deposits were assessed in aging cells with D-galactose induction. Cells expressing amyloid plaque deposits will show a brown color in their cytoplasm. The surrounding healthy cells are blue in color and there is no amyloid plaque deposition. The effect of treatment on amyloid plaque deposits on subjects who were given purple sweet potato water extract and subjects who did not get purple sweet potato extract by immunohistochemical examination observed using a 400x magnification microscope is presented in Figure 1.

DISCUSSION

Purple sweet potato water extract lowers NFkB levels

The administration of purple sweet potato water extract in this study caused the mean levels of NFkB in rats with D-galactose induction to be significantly lower than the group that did not receive purple

TABLE 1. Differences in brain tissue NFkB levels in the two groups after observation

Group	Brain tissue NFkB levels (pg/mg)			Min-max	P*
	N	Average+SD	Average Difference		
Control	16	1,672.24+202.80	254.69	87.64-421.73	0.004†
Treatment	16	1,417.55+255.82			

*Dependent t-test, † significant

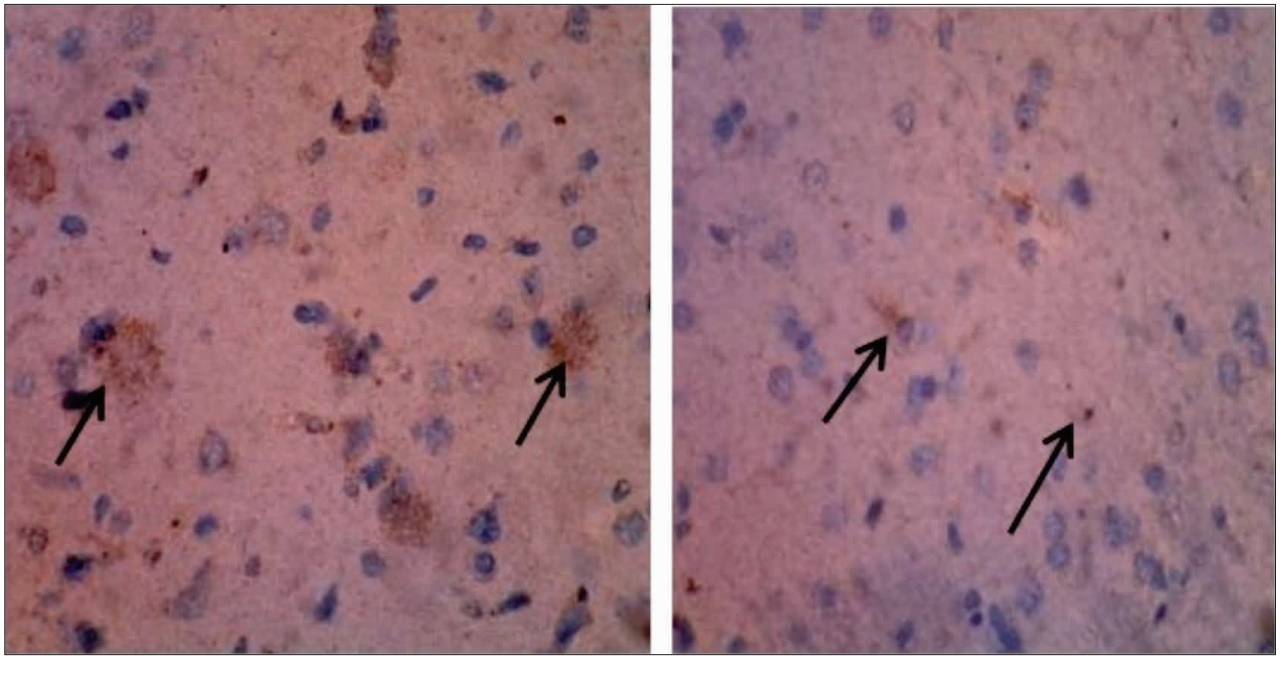

FIGURE 1. Intracellular amyloid plaques with polyclonal antibodies in the treatment group were thinner than the control group

sweet potato water extract. This may be due to the anti-inflammatory effect of anthocyanins in the aqueous extract of purple sweet potato. The results obtained in this study were similar to previous studies in the form of giving black soybean extract containing anthocyanins to reduce TNF- levels in D-galactose-induced dementia models of mice through the role of NF-κB in its cellular mechanism (11). Administration of anthocyanins reduced TNF- levels of brain tissue in the hippocampus and cortex areas in rats given D-galactose with anthocyanins compared to those given D-galactose alone. This indicates the effectiveness of anthocyanin against D-galactose-induced neuroinflammation in rat brain. Anthocyanins from black soybean decreased oxidative stress index and inflammatory markers including reactive microglia, NF-κB expression, JNK phosphorylation and pro-inflammatory cytokine production. These results further correlated with a significant decrease in pro-apoptotic proteins, an increase in pro-survival signaling and an increase in neuronal survival (8).

Administration of anthocyanin from Lycium ruthenicum Murr can suppress the activation of NF-B and other inflammatory factors in D-galactose-induced mice. D-galactose activates NF-B through interaction with RAGE (12). D-galactose significantly increased NFkB activation in the hippocampus and cortical areas. Activation of NFkB then causes the activation of various inflammatory markers so that eventually neurodegeneration occurs. Administration of anthocyanins reduced NFkB levels and decreased pro-inflammatory proteins such as iNOS and TNF- in the hippocampus and cortex in the brains of rats receiving anthocyanin and D-galactose induction compared to those receiving D-galactose induction alone. This study demonstrated the effective role of anthocyanins in suppressing D-galactose induced neuroinflammation (12).

Other studies assessing the anti-inflammatory effect of anthocyanins on microglia cultured cells of BV2 and C8-B4 mice showed anthocyanins were able to significantly suppress the pro-inflammatory proteins iNOS and COX2 in response to stimulation with lipopolysaccharide, a bacterial cell wall component known to induce an inflammatory response (11). Modulation of the p38MAPK/ERK/Akt signaling pathway correlates with decreased NF-κB which prevents translocation to the nucleus and is able to mediate many pro-inflammatory genes. Anthocyanins modulate the inflammatory response through decreased expression of TLR4 which is located upstream of NF-κB activation. Anthocyanins can increase amyloid beta peptide phagocytosis and reduce microglial inflammation through inhibition of ERK1/2.

Purple sweet potato extract reduces amyloid plaque deposits

Brain tissue in rats receiving purple sweet potato extract did not show any abnormalities in histology. There was no apparent deposition of amyloid plaques in the treatment group compared to the control group. Meanwhile, the brain tissue of the control group that received D-galactose induction showed changes in histopathological picture in the form of degeneration of neurons surrounded by microglia cells.

In vitro study on PC12 cells subjected to Aβ toxicity to assess the effect of anthocyanins on purple sweet potato extract as a strong antioxidant. The results showed that pretreatment of PC12 cells with purple sweet potato extract decreased Aβ-induced toxicity, decreased ROS formation and lipid peroxidation. In addition, purple sweet potato extract also inhibited cell apoptosis triggered by Aβ characterized by inhibition of DNA fragmentation and caspase 3 activation. The study concluded that purple sweet potato extract could protect PC12 cells from A-induced damage by inhibiting oxidative damage, intracellular calcium influx, mitochondrial dysfunction and ultimately inhibit cell apoptosis (13).

A similar study investigated the neuroprotective effect of cyanidin, a natural flavonoid by inhibiting Aβ-induced apoptosis by improving mitochondrial membrane potential by increasing the expression of Bcl-2 protein, inhibiting the accumulation of ROS and SOD. This study shows that cyanidin as a form of anthocyanin can suppress the cytotoxicity of Aβ by preventing oxidative damage and thus inhibiting apoptosis (12).

This study succeeded in proving that the administration of purple sweet potato water extract could suppress inflammation which was indicated by lower levels of NF-κB in the treatment group than the control group. The neuroprotective effect of purple sweet potato extract was assessed from the thinning of amyloid plaque deposits in the treatment group compared to the control group.

CONCLUSIONS

The results of this study strengthen the theory of chronic inflammation as a factor that plays an important role in the pathogenesis of dementia. This is indicated by the finding of lower levels of NF-kB in d-galactose-induced rats given purple sweet potato extract than those not given purple sweet potato extract and deposits of amyloid plaque in d-galactose-induced rats given purple sweet potato extract more. thinner than those who were not given purple sweet potato extract thinner.

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