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Purple sweet extract activates autophagy through enhanced mTOR expression in D-galactose-induced dementia rats

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***Abstract*---**The neuropathology of Alzheimer's dementia (AD) is characterized by the buildup of amyloid beta peptide plaques and tau protein in the brain that causes cognitive deficits. The mammalian protein kinase target of rapamycin (mTOR) plays an essential role in controlling the balance of protein synthesis and degradation through autophagy. This study was conducted to determine the role of purple sweet potato water extract in activating autophagy through the mTOR pathway. The study used a randomized posttest-only control group design where 20 male Wistar rats were randomized into the treatment group, and the control group was then induced with d-galactose 100mg/kg BW/day for four weeks. The treatment group was given an aqueous extract of purple sweet potato at 200mg/kg BW/day while the control group was given aquabidest solution for six weeks. The independent t-test showed that the mean mTOR expression in the treatment group (30.30±4.42) was significantly higher ($p<0.05$) than in the control group (16.89±2.77). Purple sweet potato extract to d-galactose-induced mice increased the autophagy process, characterized by increased mTOR expression. The research implies that the purple sweet potato extract administration can increase the

autophagy process and provide neuroprotection effects in rats by d-galactose induction.

***Keywords*---**autophagy, dementia, d-galactose, mTOR

Introduction

As the reach of health services increases, there is an increase in life expectancy and cases of diseases associated with neurodegenerative conditions. 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, making it 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 its early phase is characterized by a progressive decline in memory function and then develops involving several cognitive domains and behavioral changes (Widyastuti *et al.*, 2020).

The neuropathological sign in the AD brain is the accumulation of amyloid beta peptide plaques and neurofibrillary tangles (NFTs) of phosphorylated tau. Accumulation of beta-amyloid and soluble tau protein causes plaque formation and cognitive deficits. The mammalian target of rapamycin (mTOR) is a protein kinase that plays an essential role in controlling the balance between protein synthesis and degradation. mTOR integrates growth factors, nutrition, energy level, and stress signals, further controlling ribosome biogenesis, transcription, translation, and macroautophagy (Oddo, 2012).

Autophagy is a natural cellular defense process to eliminate abnormal proteins and damaged or degraded organelles. The neuronal autophagy-lysosomal system disorders are known to be associated with various AD symptoms. Dysfunction of the neuronal autophagy-lysosomal system can lead to ineffective protein clearance, resulting in neuronal cell death (Shacka, Roth, and Zhang, 2008). mTOR activation induces Aβ production and aggregation by directly inhibiting the autophagy/lysosomal system. On the other hand, many in vitro studies have suggested that mTOR activity is upregulated when cells are induced with Aβ by activating the PI3K/Akt pathway (Putu Eka Widyadharma *et al.*, 2020).

Purple sweet potato has been studied in several countries and is known to contain high levels of flavonoids, especially anthocyanins. Anthocyanins, as a natural antioxidant in purple sweet potato (Ipomoea batatas L) from Bali cultivars, have antioxidant effects by suppressing the production of malondialdehyde (MDA) in vivo and inducing endogenous antioxidants (Jawi, Yasa, and Mahendra, 2016). 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 (Hwang *et al.*, 2011).

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 study aging and aging-related neurological disorders, including DA (Shwe *et al.*, 2018). Administration of excessive doses of exogenous D-galactose beyond average 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 (Rehman *et al.*, 2017). In dementia model mice, a gradual increase in the number of Aβ plaques has been reported (Arvanitakis, Shah, and Bennett, 2019). A recent study reported the ability of anthocyanin-rich extracts to modulate autophagy to clear toxic protein aggregates from the intracellular space, thereby preventing neuronal death. Anthocyanins significantly increase autophagosome turnover and activate the mammalian target of rapamycin (mTOR), one of several regulators of the autophagy pathway (Poulose *et al.*, 2017). This research is expected to further study the benefits of anthocyanins in the pathogenesis of autophagy dementia as assessed from the expression of mTOR.

Method

This research is an experimental study with a posttest control group design conducted at the Pharmacology Laboratory, Faculty of Medicine, Udayana University. This research was conducted at the Pharmacology Laboratory of the Faculty of Medicine, Udayana University, for six months starting in February-July 2021. The ethical license No.1032/UN14.2.2VII.14/LT/ 2020 from the ethics committee of the Faculty of Medicine, Udayana University/ Sanglah Central General Hospital Denpasar.

The sample population in this study was 20 samples of male Wistar rats aged 12-14 weeks weighing 200-300grams kept in the Pharmacology Laboratory of the Faculty of Medicine, Udayana University, divided into two groups, control and treatment with exclusion criteria of sick and hyperactive rats.

Analysis and presentation of data with mTOR expression data expressed on a numerical scale, normality test was carried out using the Shapiro Wilk test, and the data were normally distributed; then, an independent parametric t-test was performed to find differences in the two groups. The level of significance with $p<0,05$ and 95% confidence interval

Result and Discussion

Experimental research with a posttest control group design was carried out from June to August 2021. The subjects of this study were 20 rats divided into two groups, namely the control group and the treatment group. The control group received d-galactose and aquabidest, while the treatment group received d-galactose and purple sweet potato extract. Purple sweet potato extract in the treatment group was given two weeks before d-galactose induction. Induction of d-galactose at 100 mg/kg/day dose via intraperitoneal injection at 08.00 am. The purple sweet potato extract was administered orally at a dose of 200mg/kg BW/day via a nasogastric tube once a day at 08.00 am.

The effect of giving purple sweet potato water extract on mTOR expression in D-galactose-induced rats was measured by comparing the mTOR expression in the treatment and control groups. A normality test was performed on the mTOR expression data for both groups using the Shapiro-Wilk normality test. The normality test showed that the data were normally distributed with p>0.05. The homogeneity test of the Levene test showed homogeneous data, followed by a parametric independent t-test which aimed to assess the difference in the mean mTOR expression between the treatment and control groups. The level of significance was measured with a p-value <0.05. The results of the analysis of brain tissue mTOR expression in the two groups after observations are presented in Table 1.

Table 1
Differences in brain tissue mTOR expression in the two groups after observation

Brain tissue mTor expression (%)					95% CI		p
Group	N	Mean±SD	Range (Min-Max)	Average difference	Min	max	
Control	10	16.89±2.77	11.22-119.77	-13.4	-16.87	-9.93	<0.001*
Treatment	10	30,30±4.42	23.68-38.41				

*Chi-Square

The examination of mTOR levels in the control group averaged 16.89±2.77%, while in the treatment group, it was 30.30±4.42%. The results of the independent t-test analysis in table 4.1 showed that the water extract of purple sweet potato caused the mean mTOR levels in the treatment group to be significantly higher than in the control group (p<0.05).

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

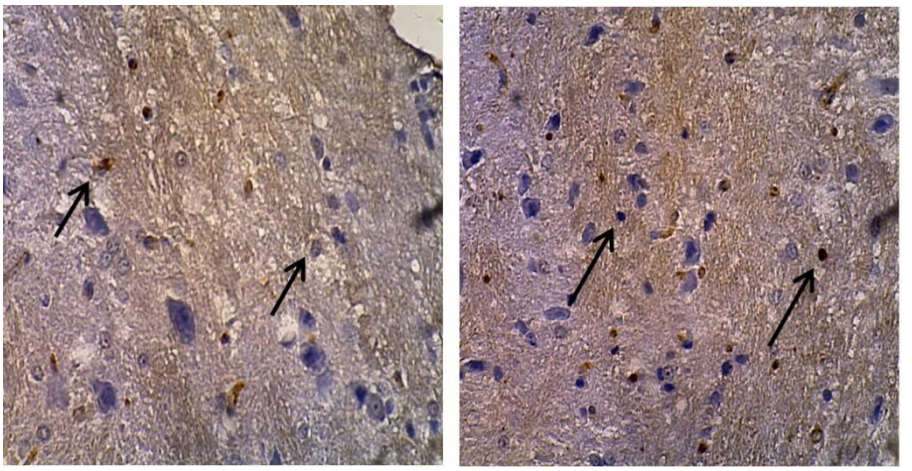

Figure 1 Expression of mTOR with polyclonal antibodies in the treatment group was higher than in the control group

The purple sweet potato aqueous extract in this study caused the mean mTOR expression in D-galactose-induced rats that received purple sweet potato extract to be significantly higher than the group that did not receive purple sweet potato aqueous extract. This is possible because of the antioxidant effect of anthocyanins in the water extract of purple sweet potato. The results obtained in this study were similar to Evans *et al.* (2011), that administration of purple sweet potato extract inhibits the mTOR pathway and activates the autophagy pathway in flies. The mTOR signaling pathway promotes oxidative metabolism and plays a role in apoptosis and autophagy. Inhibition of the mTOR pathway was shown to significantly extend the lifespan of flies through the upstream AKT-1, PI3K, and mTOR genes and downstream 4E-BP in the treatment group that received purple sweet potato extract (Han *et al.*, 2021)

Autophagy is the process by which autophagosome proteins or organelles are engulfed into vesicles and fused with lysosomes to form autophagosomes and remove their contents. Purple sweet potato is rich in anthocyanins, and other phenolic compounds are known to have potential antioxidant activity to delay aging (Hsieh *et al.*, 2021). The results are consistent with Liu's report showing that Rhodiola Rosea extracts decreased bladder cancer cell growth by inhibiting the mTOR pathway and prolonging lifespan (Yang *et al.*, 2019). As a cellular process, autophagy plays an indispensable role in cell survival due to conditions of nutrient deficiency and abnormal cell growth. The reduction of phagosomes in autophagy has been widely reported in the aging process (Nazio *et al.*, 2013). Lysosomes are organelles that break down proteins, nucleic acids, polysaccharides, and other biological macromolecules, reflecting the degree of autophagy. Expression of autophagy-related genes was increased, and intestinal epithelial cell lysosomal production was significantly increased in flies treated with purple sweet potato extract, indicating that the autophagy pathway was activated (Anuradha Bhukel, Frank Madeo, 2016)

The ability of anthocyanin-rich extracts to modulate autophagy was reported by studies (Poulose *et al.*, 2017). In addition to proteasomal degradation, autophagy plays an essential role in clearing toxic aggregates and misfolded proteins from the intracellular space to prevent neuronal death (Poulose *et al.*, 2017; Juan *et al.*, 2019). Acai fruit helped stimulate autophagy in vitro and in vivo. This extract significantly increased autophagosome turnover and increased activation of mTOR, which is one of several regulators of the autophagy pathway. These results were confirmed in vivo in the brains of aged rats treated with acai pulp extract showing upregulation of autophagy, namely mTOR activation (Poulose *et al.*, 2017). Supplementation of tart cherries in aged mice also showed similar results in increased autophagy markers in the hippocampus (Thangthaeng *et al.*, 2016). Another study showed that purple sweet potato extract significantly increased autophagy markers in the hippocampus of rats fed a high-fat diet in the form of AMP-activated protein kinase (AMPK) activation. These changes correlate with reduced hippocampal neuronal apoptosis and a significant increase in brain neurotrophic factor BDNF (Juan *et al.*, 2019). Overall, anthocyanins and anthocyanin-rich extracts can modulate protein aggregation and autophagy to ameliorate impaired protein homeostasis in neurodegenerative diseases, although further data confirmation is needed (Winter and Bickford, 2019).

The AKT/mTOR signaling pathway is a classic anti-apoptotic pathway involved in multiple cellular activities and closely related to cell survival, cell cycle, cell proliferation, and apoptosis. Several recent studies have shown that regulating this pathway can prevent or improve neurodegenerative diseases (Oddo, 2012). Overexpression of mTOR via upstream signaling cascades such as PI3-K/Akt, GSK3, AMPK, and IGF-1 is also known to cause AD. Deregulating this pathway in various neurodegenerative diseases leads to hyperactivation of mTOR, leading to tau hyperphosphorylation and the formation of NFTs in AD. mTOR also contributes to the production and aggregation of Aβ plaques by directly inhibiting autophagy. An accumulation induces tau hyperphosphorylation and mTOR activation in AD development. The reciprocal relationship between mTOR-tau-Aβ is attractive for a possible therapeutic approach. An important aspect of therapeutic development is inhibiting AD development without interfering with normal signaling pathways (Mueed *et al.*, 2019).

The expression of mTOR plays a crucial role in controlling protein homeostasis by regulating the synthesis and degradation of proteins directly related to learning and memory. It was later confirmed in mammals that bilateral infusion of rapamycin into the auditory cortex does not interfere with the maintenance of new memories but causes impaired long-term memory consolidation. Inhibition of mTOR activity is detrimental to basal synaptic plasticity. However, there is also evidence to suggest that mTOR hyperactivity also has a detrimental effect associated with deficits in the potentiation of long-term memory and hippocampal-dependent memory (Oddo, 2012)

CNS neurons have minimal regenerating ability, so they must have efficient oxidative metabolism to prevent cell death. Proper synapse function is highly dependent on mitochondria, endoplasmic reticulum, lysosomes, and the axonal influx of calcium ions and neurotransmitters. Mitochondrial dysfunction is associated with disruption of the Krebs cycle and biochemical pathways and

reduced availability of ATP. Mitochondrial and lysosomal changes in morphology and signaling can initiate neurodegeneration and death of susceptible neurons. Autophagy is not only a feature of neurons but also occurs in astrocytes, oligodendrocytes, and microglia in aging and neurodegenerative disorders (Stacchiotti and Corsetti, 2020).

Neurodegenerative diseases are multifactorial, and many pathways must be considered. Not only autophagy but also necrosis and apoptosis contribute to neuronal cell death. Natural compounds capable of limiting neurodegeneration in vitro may not be effective in vivo, and some may act naturally or synthetically (Stacchiotti and Corsetti, 2020). The autophagy mechanism plays a role in reducing abnormal protein deposition and reciprocal cleaning of organelles in neurons. Mizushima (2018) stated that measuring human autophagy flux is still impossible. As a result, direct studies of the efficacy of natural substances on autophagy are still lacking due to inadequate quantitative methods (Mizushima, 2018). Further confirmation of the safe use of bioactive compounds to prevent or limit unavoidable neurodegeneration is needed. An understanding of the pharmacokinetic mechanism of action and the non-specific effects of natural compounds is needed in the treatment of neurodegenerative diseases (Stacchiotti and Corsetti, 2020)

Conclusion

The results of this study strengthen the theory of chronic inflammation as a factor that plays an essential role in the pathogenesis of dementia. This was shown from the finding that the mTOR expression in rats with d-galactose induction given purple sweet potato extract was higher than in those not given purple sweet potato extract. This indicates that the administration of purple sweet potato extract can increase the autophagy process and provide a neuroprotective effect in rats by d-galactose induction. Further research is needed on the neuroprotective potential of purple sweet potato extract related to increasing understanding of the pathogenesis of AD in preclinical models.

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