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Research Paper

PROTECTIVE EFFECT OF ALPINIA GALANGA AGANIST D-GALACTOSE INDUCED BRAIN AGING

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D-galactose induced neurotoxicity is well known model for studying aging and related oxidative damage and memory impairment. Aging is a biological process, characterized by the gradual loss of physiological functions by unknown mechanism. This study attempted to access the neuroprotective effect of Alpinia galanga on the senescent rat induced by D-galactose (D-gal). The rats in the experiments were orally administered with Alpinia galanga (500 mg/kg), for four weeks. Alpinia galanga fed rat showed higher activity by increase in fall off time in rotarod test, showing higher attention in visual placement test, increase in sniffing time for the new object in novel object recognition test, and significantly improved learning and memory ability in Morris water Maze tests compared with D-galactose treated rat. Alpinia galanga significantly increased superoxide dismutase (SOD), Catalase (CAT), Glutathione (GSH), Total Protein Content (TPC) activity and decreased the malondialdehyde (MDA) level. Alpinia galanga also attenuated enhanced acetylcholine esterase enzyme level in D-galactose senescence rat. Present study highlights the protective effect of Alpinia galanga against D-galactose induced behavioral and biochemical parameters in rat. These results indicated that Alpinia galanga has the potential to be a useful treatment for cognitive impairment. In addition, the memory enhancing effect of Alpinia galanga may be partly mediated via enhancing endogenous antioxidant enzymatic activities.

Keywords: Alpinia galangal, Cognition deficit, Aging, D-galactose

INTRODUCTION

Aging is a slow and gradual biological process, associated with various morphological and biochemical changes in biological system is discussed by Toren (2003). Memory decline is a characteristic of aging and age-related neurodegenerative disorders which lead to a progressive loss of cognitive function, especially in spatial memory is reported by Barnes *et al.* (1980). Cognitive abilities show at least a small decline with advanced age in many, but not all, healthy individuals is reported by Diane Howieson (2015).

Researchers have shown that the dopamine D2 receptor is linked to the long-term episodic memory, which function often reduces with age and due to dementia. Researchers could see that the D2 system was positively linked to episodic

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memory, but not to working memory. Researchers could also see that the D2 system affects the functioning of the hippocampus in the brain, long linked to long-term episodic memory discussed in Recent Research on Aging and Regeneration in the Brain (2016).

Among the countless theories proposed for aging, the formation of ROS is an important step leading to neuronal death in a variety of age-related neurodegenerative disorders including Alzheimer's disease and Parkinson's disease reported by Olanow (1993) and Cini *et al.* (1994). The accumulation of free radicals progressively damages brain structure and function was reported by Finkel and Holbrook (2000).

D-galactose is a normal reducing sugar in the body. At its normal level, it is usually converted in to glucose by galactose-1-phosphate, uridyltransferase and galactokinase. *D*-galactose at higher levels is converted to aldose and hydroperoxide under the catalysis of galactose oxidase, resulting in the generation of a superoxide anion and oxygen-derived free radicals by Song *et al.* (1999). Many investigations using rodents have demonstrated that the injections of D-galactose can lead to excessive formation of ROS, neuronal damage and a significant decline in learning and memory capacity discussed by Zhang *et al.* (1990) and Lu *et al.* (2006).

Rodent chronically injected with D-galactose has been widely used as an animal aging model for brain aging or anti-aging pharmacology research is reported by Wei *et al.* (2005). D-galactose induced brain aging is similar to normal aging. D-galactose (60 mg/kg) administered for 4 weeks causes aging by Kakkar (1984).

Alpinia galanga rhizome belongs to the family Zingiberaceae. As per Ayurveda, all parts of the plant are medicinally important. In folklore medicine, *Alpinia galanga* is reportedly used in treating Rheumatism, bronchial catarrh, bad breath, ulcers, whooping cold in children, throat infections, control incontinence and fever was published in Compendium of Indian Medicinal Plants (1994). The ethanolic extract of *Alpinia galanga* root has been reported to possess acute and chronic inflammation (Aisha Karim Khan and Darakhshan Haleem, 2011). Moreover methanolic and aqueous extract of *Alpinia galanga* has been found to have hypoglycemic activity is discussed by Kaushik Yadav (2011). The methanolic extract of A.galanga reported anti-platelet activity. Ethanol extracts of A.galanga act against Staphylococcus aureus 209P and Escherichia coli NIHJ JC-2 (Ohkawa *et al.*, 1979). Methanolic extract of *A.galanga* rhizome showed moderate anti-bacterial activity against all experimental bacteria except *E.coli* is reported by Caichompoo (1999). The present study has been undertaken to investigate the potential role of Alpinia galanga in the brain of D-galactose induced rats by quantifying lipid peroxidation and various antioxidant enzyme activities.

MATERIALS AND METHODS

Plant Material

The plant material (rhizome of Alpinia galanga) collected from chilakalagadda near araku region of Vishakhapatnam; Andhra Pradesh was authenticated by Dr. K Srinivas Rao, taxonomist, Andhra University. A voucher specimen is kept in SVIPS/2013/016 for further studies.

Preparation of Extract

The collected rhizomes were shade dried for 20 days and are made to coarse powder and was macerated using ethanol. As a menustrum standing for 7 days shaking occasionally. After

maceration the liquid is strained off and solid residue is (mark) pressed. Then it is clarified by filtration. The filtrate was air dried to obtain fine powder. The yield of ethanolic extract of *Alpinia galanga* is 12.5% w/w.

Acute Toxicity Study

Acute toxicity study for the ethanolic extract of *Alpinia galanga* was done according to the Organization for Economic Co-operation and Development (OECD) guidelines No. 423 (Acute toxic test method). The OECD Test guidelines are recognized world-wide as the standard reference tool for chemical testing. The low and high doses of *Alpinia galanga* were selected for treatment as per OECD guidelines (Subash *et al.*, 2011).

Animal Grouping

Albino rats were randomly divided into seven groups each of six animals.

Group 1: Adult Control: Animals received Tween 80 (0.05%, in distilled water) orally for 49 days.

Group 2: Aged Control: Aged animals received Tween 80 (0.05%, in distilled water) orally for 49 days.

Group 3: Positive Control: Animals received D-galactose (D-gal, 60 mg/kg, i.p.) once a day for 49 days.

Group 4: Adult Test: Adult animals received treatment- dose +D-Gal (60 mg/kg; i.p.) for 49 days.

Group 5: Aged Test: Aged animals received only treatment- dose orally for 49 days.

Group 6: Adult Standard: Adult animals received standard drug Donepezil dose +D-Gal (60 mg/kg; i.p.) for 49 days.

Group 7: Aged Standard: Aged animals received only standard drug donepezil dose orally for 49 days.

Tissue Homogenate Preparation

The brain tissue was washed with ice cold 0.9% saline solution. Brain was completely crushed using a syringe Plunger. The veins and large extra arteries in the brain were removed. 10 ml of ice cold 0.1 M Tris-Hcl buffer (PH 7.5) was added to the crushed brain and homogenized with Remi homogenizer to give a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatants were used for estimation of total protein (TP), malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and acetylcholinesterase (Ach) (Ohkawa *et al.*, 1979; Shukitt, 1999; and Srikumar *et al.*, 2004).

Behavioural Tests: Behavioural tests was described by Barnes *et al.* (1980), Crawley (1999) and Diane Howieson (2015).

1. Rotarod test
2. Morris water maze test
3. Object recognition test
4. Visual placement test
5. Grip strength test

Biochemical Assessment:

Estimation of Total Protein Content: (Lowry *et al.*, method 1951).

Estimation of Catalase (CAT): (Aebi, method 1974)

Estimation of Superoxide Dismutase

Estimation of Glutathione

Estimation of *Malondialdehyde (MDA) levels*

Estimation of Acetylcholinesterase

Histopathological Studies

Brains were quickly removed, preserved in 40% formalin, processed and embedded in paraffin. Four micrometer thick paraffin sections were cut on glass slides and stained with hematoxylin and eosin (H) reagents and observed by light microscope to evaluate brain injury.

Statistical Analysis

The data were analyzed by using the one way analysis of variance (ANOVA) with the Graph pad instant demo version and a p value of < 0.05 was considered as statistically significant. The mean and the standard deviation were calculated for each parameter in each group.

RESULTS

Behavioural Tests

Rota Rod Method

Table 1: Effect of Alpinia Galanga Extract on Rotarod Test for Young Animals

Groups	Control (Y)	Disease Control	Test (Y)	Standard (Y)
Time (in sec)	194 ± 3	30± 2.3	135 ± 5*	145 ± 28*

Table 2: Effect of Alpinia Galang Extract on Rota Rod Test for Aged Animals

Groups	Control (A)	Disease Control	Test (A)	Standard (A)
Time (in sec)	47± 2.7	30±2.3	44± 2.7*	45.6 ±3*12

Water Maze Test

Table 3: Effect of Alpinia Galanga Extract on Water Maze Test for Young Animals

Groups	Control (Y)	Disease Control	Test (Y)	Standard (Y)
Time (in sec)	2.496± 0.17	7.1± 0.2	2.8± 0.22*	2.39± 0.19*

Table 4: Effect of Alpinia Galang Extract on Water Maze Test for Aged Animals

Groups	Control (A)	Disease Control	Test (A)	Standard (A)
Time (in sec)	3.02± 0.19	7.1± 0.2	3.02+0.26 *	3.46± 0.38*

Object Recognition Test

Table 5: Effect of Alpinia Galanga Extract on Object Recognition Test for Young Animals

Groups	Control (Y)	Disease Control	Test (Y)	Standard (Y)
Time (in sec)	11.2±0.2	7.26±0.08	10±0.2*	10±0.3*

Table 6: Effect of Alpinia Galanga Extract on Object Recognition Test for Aged Animals

Groups	Control (A)	Disease Control	Test (A)	Standard (A)
Time (in sec)	10.7±0.19	7.2±0.08	10.5±0.3*	11.6±0.2*

Visual Placement

Table 7: Effect of Alpinia Galanga Extract on Visual Placement Test for Young Animals

Groups	Control (Y)	Disease Control	Test (Y)	Standard (Y)
Scores	2± 0.01	0.4± 0.2	1.8± 0.2*	2± 0.01*

Table 8: Effect of Alpinia Galanga Extract on Visual Placement for Aged Animals

Group	Control (A)	Disease Control	Test (A)	Standard (A)
Scores	1.6± 0.2	0.4± 0.2	1.8±0.2*	1.6± 0.24*

All values are expressed in Mean ± S.E.M of n = 6.* p<0.05, when compared to control group(one way ANOVA followed by Bonferroni's multiple comparison test.

Antioxidant Activities

Table 9: Effect of Alpinia Galanga Extract on Acetylcholinesterase Levels

Groups	Acetylcholinesterase Levels in Young Rats	Acetylcholinesterase Levels in Aged Rats
Control	0.02 ± 0.001	0.02 ± 0.0006
Disease control	0.03 ± 0.0013	0.03 ± 0.001
Test	0.022 ± 0.0014*	0.02 ± 0.0007*
Standard	0.02± 0.002 *	0.02 ± 0.0008*

Table 10: The Effect of Alpinia Galanga Extract on Brain Antioxidant Values in Young Animals

Groups	CAT	SOD	GSH	LPO
Control	4.25±0.09	11.5±0.23	15 ± 0.1	8.6± 0.5
Disease control	1.9 ± 0.03	5 ± 0.12	7.5±0.17	17.6±0.3
Test	4.0 ± 0.08	10.9 ± 0.3	1.43±0.2*	8.6 ± 0.2*
Standard	4.3 ± 0.08	11.9 ± 0.4	15 ±0.19*	9.1±0.08*

Table 11: The Effect of Alpinia Galanga Extract on Brain Antioxidant Values in Aged Animals

Groups	CAT	SOD	GSH	LPO
Control	3.3±0.06	9.4±0.2	11.3±0.15	10.5±0.18
Disease control	1.9±0.03	5.06±0.12	7.5 ± 0.17	17.6± 0.3
Test	3± 0.10	8.9 ± 0.3	10.7±0.11*	9.3± 0.08*
Standard	3.6±0.12	9.6± 0.22	11.3±0.15*	9.7 ± 0.2*

Estimation of Total Protein Content

Table 12: The Effect of Alpinia Galanga Extract on Total Protein Content in Brain

Groups	Total Protein Content in Young Rats (mg/ml)	Total Protein Content in Aged Rats (mg/ml)
Control	11.05 ± 0.12	10.3 ± 0.23
Disease control	4.47± 0.11	4.32 ± 0.12
Test	9.15 ± 0.24*	8.98 ± 0.19*
Standard	10.18 ± 0.07*	10.2 ± 0.31*

All Values are expressed as mean ± S.E.M of n = 6. * P<0.05, when compared with control group(one way ANOVA followed by Bonferroni's multiple comparison test).

Figure 1: Histopathological Studies

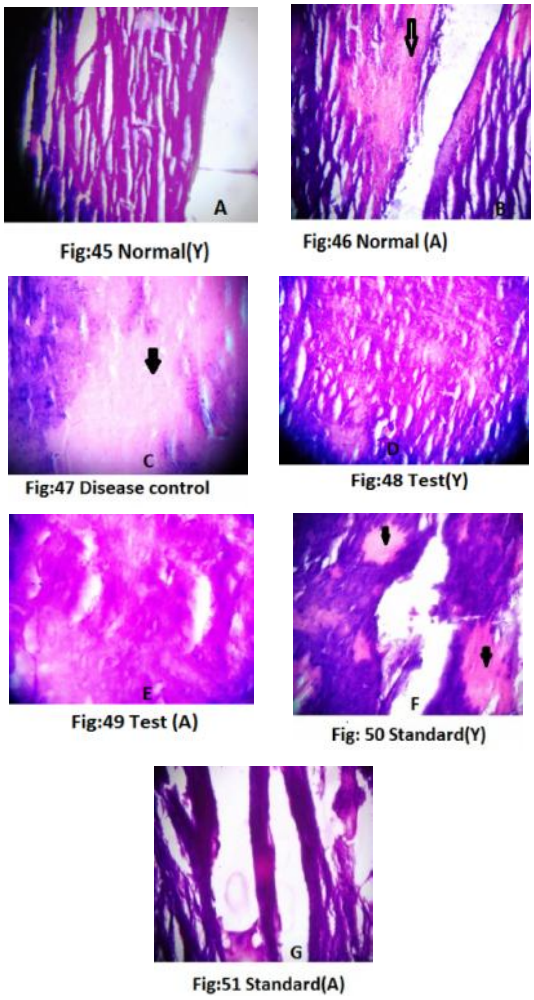

Fig:45 Normal(Y)
Fig:46 Normal (A)
Fig:47 Disease control
Fig:48 Test(Y)
Fig:49 Test (A)
Fig: 50 Standard(Y)
Fig:51 Standard(A)

DISCUSSION

D-galactose plays a prime role in the pathogenesis of aging. Various hypotheses have been put forward to explain the mechanism of D-galactose in aging including glyco metabolism block, formation of Advanced Glycation End product (AGE), and free radical injury with the evidence of increase levels of malondialdehyde, decrease in SOD, glutathione peroxidase, catalase activity (Cui *et al.*, 2006). D-galactose administration mimics some characters of cognitive dysfunction and oxidative damage; therefore, it is gradually accepted by researchers

as an inducing agent for degenerative brain disorders like Alzhemeir's disease. Aging affects number of process like memory, learning and other cognitive abilities such as; thought process, abilities to activate and focus attention. In this study, Intraperitonial (i.p) administration of D-galactose for six weeks caused impairments in memory function and cognitive ability in rat.

The free radicals generated from oxidation of D-gal over run the capacity of cells to clean them. This consequently causes the chain reactions of lipid peroxidation (LPO). The end products, such as MDA, from this combines proteins with phospholipid and leads to the injuryof cellular membrane and impairment of central nervous system (Cini *et al.*, 1994).

Still now allopathy system of medicine is silent for the drugs used in aging. Therefore the attention of the researchers is concentrated for finding some alternative therapy for it. Plants like *centella asiatica* (Anil Kumar *et al.*, 2009) *cordyceps militaris* (Zhang *et al.*, 1990) *diosgenin ameliorates* (Chang *et al.*, 2009) *Ocimum sanctum*etc., are useful for the brain aging activity. This motivated the author to evaluate the protective effect of *Alpinia galanga* and to give scientific rational for folklore use of it.

Rotarod is a measure of neuromuscular co-ordination, fatigue, and learning . Rotarod performance has often been used as a method to study the effects of drugs on neuromuscular co-ordination (Barnes *et al.*, 1980), a measure of cerebral function (Calderini, 1983) and a measure of motor learning (Hooge and De Deyn, 2001). The accelerating rotarod is better at detecting drug effects and a better model for motor learning. The Procedure as described here is a sensory motor learning task. The latency for the animal to fall off the rod will increase over trials and over days as the animal becomes more proficient with maneuvering on the apparatus and can stay on longer-galactose (60 mg/kg) significantly induce behavioural impairment in the rotarod test indicated by decrease in the fall off time. Oral administration of *Alpinia galanga* (500mg/kg) extract showed increase in the fall off time in both young and aged rats compared to D-galactose induced indicates the protective effect.

Studies of learning and memory play an important role in the study of neurological disorders with cognitive impairment, such as schizophrenia and age related disorders like Alzheimer's disease. The most common spatial navigation task is the Morris water maze.

D-galactose at a dose of 60 mg/kg could significantly induce behavioral impairment by increasing the latency time to reach the hidden platform during the Morris Water Maze test (MWM), which has been regarded as one of the most frequently used laboratory tools in spatial learning and memory of neurobiology and neuropharmacology studies. Morris water maze typically consists of a series of spatial learning acquisition training and spatial accuracy memory in probe trial (Hooge and De Deyn, 2001). D-galactose induced group shows increase in the latency time to reach the hidden platform. *Alpinia galanga* treated rats, shows significant ($p<0.05$) decrease in the latency time to reach the hidden platform which indicates protective effect. *Alpinia galanga* treated aged rats shows increase in the spatial learning memory which was evident by decreased latency time to reach the hidden platform.

The effect of aging upon cognition in rats, assessed by Object Recognition Test (ORT) by Saida Haider *et al.* (2011). It is based on comparison of sniffing time for both objects (old

and new) by both test groups (young and aged) .The results showed a significant decrease in the sniffing time for new object, indicating a cognitive impairment in D-galactose induced rats. In ORT, the D-galactose induced group shows a significant ($p<0.05$) decline in sniffing time for new object as compared to controls. Oral administration of *Alpinia galangal* (500 mg/kg) extract showed significant increase in the sniffing time for new object as compared to D-galactose group. Aged test group rats shows increase in the sniffing time for new object as compared to D-gal group. Discrimination index was calculated by comparing difference in sniffing time for new and old object. Discrimination index indicates an increase in spatial learning and memory by the test rats.

Visual placement test is used to know the attention and memory of the animals, by observing the stretching the body and profency of looking down. D-galactose induced group scores less (0 or 1) indicates the decrease in the profency of looking down compared to control group. *A.galanga* treated group scores (2) indicates the increase in the stretching and profency of looking down shows increase in cognitive attention. Donepezil (std) group Scores 2. *Alpinia galanga* extract shows increase in the learning and memory abilities by scoring more compared to induced group, and scores equally as standard (Donepezil) group.

Injection of D-galactose could induce senescent-like symptoms in animals, such as abnormal alterations in biochemistry markers, retrograde changes in neural cells and memory impairments (Ho *et al.*, 2003). As mentioned in the previous study, chronic systemic

D-galactose exposure would induce memory loss, neurodegeneration, and oxidative damage in rat (Cui *et al*., 2006). Our results were in agreement with these findings.

ROS became an active field in aging research because of their potential involvement in many degenerative processes and in many neurodegenerative diseases such as aging (Alzheimer's and Parkinson's diseases) (Harman, 1992). These ROS can be scavenged by endogenous antioxidants including CAT, SOD and GSH. MDAis a by-product of lipid peroxidation due to excessive free radical generation and is widely used as a biomarker of oxidative stress (Cini *et al*., 1994).

In this study, the activities of CAT, SOD and GSH in the brain showed a significant decline in D-galactose group rat compared to normal group rat. Treatment with *Alpinia galanga* for four weeks, improve the levels of CAT, GSH and SOD. In addition, an obvious enhancement of the level of MDA was shown in the D-galactose group rat, and it could be significantly reduced after 42 days oral administration of *Alpinia galanga* extract. It may be due to the ROS scavenging action of *Alpinia galanga* via increasing the activities of CAT, SOD and GSH, consequently decreasing lipid peroxidative damage.

Aging is associated with intellectual malfunction and subsequent decline in cognitive, behavioral and motor function. Increased levels of AChE in aging patient has led to the hypothesis that cognitive decline is related to cholinergic degeneration.Therefore promising approach for treating aging (age related disorders like AD) is to enhance Acetylcholine concentration in the brain (Sridharamurthy *et al.*, 2012). This rise in enzyme level leads to rapid cleavage of Acetylcholine and there by reduces concentration and turnover of Ach. A significant inhibition of AChE activity has been found in the rats treated with

Alpinia galanga 500 mg/kg (b.w.) for 42 days. Thus, the plant extract is found to have inhibitory effect on cognitive declination associated with aging.

In aging process induced a decrease of total protein, cholesterol and phospholipids content (Calderini, 1983). D-galactose group shows significant ($p<0.05$) decrease in total protein compared to control. *Alpinia galanga* treated group shows significant increase in the total protein content in both young and aged rats.

Donepezil is a specific noncompetitive reversible inhibitor of acetylcholinesterase (AChE) (Sugimoto *et al.*, 2000), and appears to exert its therapeutic effect by enhancing cholinergic function. By inhibiting the hydrolysis of acetylcholine produced by AChE, donepezil increases acetylcholine concentrations, thus enhancing cholinergic function. The dementia progresses, fewer cholinergic neurons remain functionally intact, and the effects of donepezil may be lessened. Donepezil exhibits a relatively high degree of selectivity for Neuronal AChE. Donepezil (cholinesterase inhibitors) can attenuate cognitive disturbances in patients with mild to moderate aging and are currently used as symptomatic treatments (Ho *et al.*, 2003; and Cheang *et al.*, 2006).

Phytochemical analysis of *Diosgenin ameliorates, Ocimum sanctum* and sweet potato shows the presence of Flavonoids and steroids phytochemicals which act as a powerful antioxidants and anti-aging agents (Jeong *et al.*, 2005; and Verbeek *et al.*, 2005)

In our plant Extract *Alpinia galanga* rhizome also having flavonoids and steroids. The protective effect of *Alpinia galanga* may be due to presence of the vital nutrients in it. In the present study, it reveals that treatment with *A.galanga* could restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals and neuronal damage.

Since natural products contain many constituents, the efficacy of the combination of natural products with Western medicine appears to fluctuate. If the combination of natural product with Western medicine does not result in interaction and possesses synergistic effect, the combination of natural product with Western medicine is to be followed. In order to decrease the fluctuation of efficacy by the combination use, it is necessary to separate the active components from the crude extract and standardize the content of active components in the extract.

CONCLUSION

In this study it concluded that, our plant Extract *Alpinia galanga* rhizome shows protective effect in brain aging. The protective effect of *Alpinia galanga* due to presence of flavonoids and steroids in it. It reveals that treatment with *A.galanga* could restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals and neuronal damage.

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