NEUROPROTECTIVE EFFECT OF AMORPHOPHALLUS CAMPANULATUS IN STZ INDUCED ALZHEIMER RAT MODEL

Dong Chen*
Department of Neurosurgery Tian Jin Huan Hu Hospital, No.122 QiXiang Tai Road, He Xi District, Tian Jin 300060, P.R. China

*Corresponding Author E-mail: chendong0601@hotmail.com

Abstract

Background: The present investigation deals with the assessment of neuroprotective effect Amorphophallus campanulatus (AC) tuber in alzheimer diseased (AD) rat and also postulates its possible mechanism of action.

Material and Methods: AD was induced by administering streptozotocin i.e. STZ (3 mg/kg, ICV) day one and 3rd day after surgery. Surgery was performed on anesthetized rats by the help of stereotaxic apparatus. STZ induced AD rats were treated with petroleum ether extract of AC (100, 200 and 500 mg/kg, p.o.) for 14 days. Effect of AC tuber in AD rats were assessed by estimating the alteration in the behavior (Y maze apparatus and single trial passive avoidance), biochemical parameter in the brain tissue {Oxidative stress parameters (SOD, CAT and LPO), amyloid β peptide (Aβ) and acetylcholinesterase (AchE)} and histopathological study of brain tissue.

Result: Treatment with AC shows significant (p<0.01) increased in the % of alteration in the behavior and step through latency in Y maze task and single trial passive avoidance test compared to AD rats. AC significantly (p<0.01) decreases the Aβ1-40, Aβ1-42 peptides and AchE in the brain tissue compared to AD rats. Whereas, treatment with AC significantly reduces the oxidative stress level in AD rats. Histopathological study reveals that treatment with AC extract reduces the amyloid plaque formation in the brain tissue of AD rat.

Conclusion: The present study concludes the neuroprotective effect of AC extract in AD rats by reducing oxidative stress, Aβ and AchE in the brain tissue.

Key word: Amorphophallus campanulatus, Alzheimers, amyloid β peptide (Aβ) and acetylcholinesterase (AchE), Oxidative stress.

Introduction

Alzheimer is associated with neuronal degeneration, characterized by memory loss and altered behavior. Many pathological conditions like increased oxidative stress, amyloid β (Aβ) plaque formation, neuroinflammation results in AD (Grosgen et al., 2010). Oxidative stress develops due to excess of reactive oxygen species (ROS) which overexpress Aβ around the neuron that alters the cellular function and thereby triggers the neurodegeneration (Smith et al., 2010; Zawia et al., 2009; Coppieters and Dragunow, 2011). Literature reveals that alterations in the neurotransmitters level (glutamate, GABA, dopamine) are responsible for neurodegeneration or neurodegenerative disorders (Chilumuri and Milton, 2013). Various reports suggested that drugs having free radical scavenging activity possess neuroprotective effect (Uttara et al., 2009). In the recent years use of complementary medicine like herbs has increased in the management of chronic disorder. Herbs are the source of mostly drugs which plays the role in the development of drug (Hedrick, 1972).

Amorphophallus campanulatus (Araceae) is widely available all over the Asian and African countries. Traditionally AC was used for the management of tumors, inflammations, fatigue, anemia, bronchitis etc. (Nair, 1993). Moreover, various studies suggested that AC posses Antitumor, analgesic, antioxidant and immunomodulatory activities (Angayarkanni et al., 2007; Prathibha et al., 1995; Shilpi et al., 2005; Tripathi et al., 2008; Tripathi et al., 2010).

Materials and Methods

Collection and preparation of plant extract

Fresh Amorphophallus campanulatus tuber was collected from the supplier. The tuber was chopped into small pieces and dried it under shade at room temperature. Dried tuber was coarsely powdered and kept it in to a plastic container with petroleum ether for 72 hrs. The extract was concentrated using a rota vapor at reduced temperature and pressure in order to remove the solvent completely. It was dried and kept in a desiccators till experimentation (Yield 7.4% w/w).
Animals

Healthy male wistar rats (250-300 g) at about 8 weeks of age were used for the pharmacological screening in the present study. The animals were housed at 25 ±2°C temperature, 12 h light/dark cycle and 60 ± 5 % of relative humidity. Rats were feed with standard diet and water ad libitum. Protocols of the present investigation for all the animal studies were approved by the Institutional Animal Ethical Committee.

Acute toxicity study

The acute toxicity study was performed for petroleum ether extract of *Amorphophallus campanulatus* according to the OECD 423 guidelines. The extract at different doses of 5, 50, 300 and 2000 mg/kg, p.o., were administered to the rats and observed closely for 14 days. All the rats were observed for behavior changes and mortality at the end of experiment. The extract was found safe up to 2000 mg/kg.

Induction Alzheimer’s

Male wistar rats (250-300 g) were anesthetized by IP injection of a combination of ketamine and xylazine (100 and 5 mg/kg, respectively) and then all the rats were operated by using stereotoxic apparatus (Stoelting, USA). Pxnlos and Watson stereotoxic atlas was used for the surgery, rats scalp washed by using iodine solution, incised on midline and a hole was drilled through the skull 0.8 mm post bregma, 1.4 mm lateral from midsagittal line and 3.4 mm below the dura. Rats were divided into 5 different groups: control (Shame operated), Streptozotocin treated rat (STZ, 3 mg/kg, icv), Streptozotocin plus *Amorphophallus campanulatus* 100 mg/kg (STZ+AC 100 mg/kg, p.o.), Streptozotocin plus *Amorphophallus campanulatus* 200 mg/kg (STZ+AC 200 mg/kg, p.o.), Streptozotocin plus *Amorphophallus campanulatus* 500 mg/kg (STZ+AC 500 mg/kg, p.o.). The STZ groups received bilateral ICV injection of streptozotocin (3 mg/kg, body weight) which was dissolved in citrate buffer (pH 4.4). STZ concentration was prepared so as to deliver 5 μl/injection site of the solution. Rats in the control group received icv injection of same volume of citrate buffer as in STZ treated and the injection was repeated on day 3. AD rats were treated for 2 week with *Amorphophallus campanulatus* extract and its anti-alzheimer’s activity was assessed by using behavioral models (Y maze task and single trial passive avoidance) and biochemical parameters in the brain tissues of rats (Aβ, AchE, Oxidative Stress Parameters) (Pitsikas and Sakellaridis, 2006)

Behavioral models

Y-maze task

Spontaneous alteration behavior was recorded in as single session Y maze study for the assessment of spatial recognition memory on the 14th day after STZ administration. The Y maze was prepared with black colored Plexiglas having the dimension of 40 cm in length, 30 cm in height and 15 cm wide of each arm with a triangular shape central area. Each rat was placed at the end of arm and permitted it to move for 8 min. Successive entry in each arm was considered as alteration in behavior. Possible alterations were determined by overall number of entry in arms subtracted by two (Hritcu et al., 2012; Foyet et al., 2011).

Single trial passive avoidance test

The apparatus used for condition avoidance consisted (40/20/30 cm of length/wide/high respectively) of two compartment viz. illuminated compartment linked to a dark compartment by a decapitate door.

The floor was connected to an isolated stimulator to provide electric shocks. On the first two day of study, each rat was habituated by placing 15 min in the apparatus. Acquisition trial was performed on third day. Each rat was placed in the illuminated chamber. After a habituation time, the decapitate door was lifted and allow the rat to enter in the dark compartment.

The door was closed and about 8 inescapable single electric shock (1 mA, 1 s) was delivered. Entrance into the dark chamber was recorded as a initial latency (IL) and rats with more than 60 s of IL considered in the study. One day later, retention trial was done by placing each rat in the illuminated compartment. Step-through latency (STL) was determined by measuring the time taken by the rat to transfer from illuminated compartment to the dark one (Vallee et al., 1997).

Estimation of Biochemical parameter

Brain tissue homogenate preparation

Rats were scarified and brain dissected out, washes it thoroughly with saline solution and divided into two halves. One half of brain of each rat was homogenized instantaneously in solution containing Tris-Hcl (50 mM, pH 7.4) and sucrose (300 mM).

The tissue homogenate was centrifuged at 10000 RPM for 10 min at 4°C and the supernatant was separated for the below given biochemical estimation (Tsakiris et al., 2004). Another half of brain of each rat was used for histoplathological study.
Estimation of beta amyloid

$\text{A} \beta$ was measured in brain tissue extracts using a ELISA Kit. $\text{A} \beta$ was captured by antibody coated plates (BNT77), and detection of $\text{A} \beta$-40 and $\text{A} \beta$-42 peptides achieved with the horseradish peroxidase conjugated antibodies BA27 or BC05, respectively. Developed Plates were analyzed at an optical density of 450 nm (Bimonte-Nelson et al. 2003).

Determination of AChE activity in brain

Colorimetric method was used for the estimation of acetylcholinesterase (AchE) activity in the brain tissues by using the kit (Quimica Clinica Aplicada S.A Co., Spain) (Den Blawan et al., 1983).

Estimation of markers of oxidative stress

Superoxide dismutase (SOD) was estimated in the brain tissue of STZ treated AD rats by using riboflavin sensitized method. The alteration in absorbance was observed for 4 min at 460 nm (Arutla et al., 1998).

Level of lipid peroxidation (LPO) was estimated the method given by by Ohkawaka in the brain tissue of rats. The quantity of malondialdehyde (MDA) was estimated at 532 nm (Ohkawa et al., 1979).

Activity of catalase (CAT) in the brain tissue was assessed on the ability of catalase to oxidize $\text{H}_2\text{O}_2$. The change in absorbance was recorded for 3 min at 1 min interval at 240 nm (Beers & Sizer, 1952).

Histopathology study

Isolated brain tissue sections were stained by using congo red method. Alkaline saturated Nacl solution was used to incubate the rehydrated brain tissue sections and then deep it in to congo red solution for a specific period of time. All the sections were fixed and by using trinaocular microscope (Wilcock, 2006).

Statistical analysis

Data were expressed as mean ± SEM (n=8). Data was statistically analyzed using one way ANOVA (Dunnett post hoc test). $p<0.05$ was considered as statistically significant.

Results

![Graph](image)

**Figure 1:** Effect of *Amorphophallus campanulatus* extract on alteration in behavior in the Y-maze model in STZ treated AD rats. Values are means ± S.E.M. (n=8); *@p < 0.01 (vs. Control group), **p < 0.01 (vs. Negative control group)*
Estimation of change in behavior Parameters

Alzheimer disease was induced by STZ and after completion of treatment protocol, the effect of treatment were assessed by estimating alteration in the behavior by Y maze control and step through latency by single-trial passive avoidance test. Fig. 1 Shows the % of alteration in the behavior by using Y maze in Control (Vehicle treated), Negative Control (STZ treated), STZ+AC (100 mg/kg, p.o.), STZ+AC (100 mg/kg, p.o.) group of rats. There was significant decrease (p<0.01) in the % of alteration in behavior of Negative control group compared to control group of rats. This decrease in the % alteration in behavior confirms the AD in negative control group i.e. STZ only treated rats. Whereas, treatment with AC at the dose of 200 mg/kg and 500 mg/kg were significantly (p<0.01) increased the % of alteration in behavior compared to AD rats.

Figure 2 shows the effect of AC on STL in passive avoidance test in STZ treated AD rat. For IL, no significant difference was found between the groups. For STL, there was decrease time significantly up to 34±1.2 Sec. in STZ only treated rats compared to control group. This decreased time was recovered with the treatment significantly at the dose of AC 100mg/kg (p<0.05), AC 200 mg/kg (p<0.01) and AC 500 mg/kg (p<0.01).

Estimation of Biochemical

Estimation of β amyloid in brain tissue

β amyloid 1-40 and 1-42 peptide were estimated in the brain tissues of rats. Fig. 3 and 4 show the estimation of Aβ1-40 and Aβ1-42, these subunits of β amyloid peptide in brain tissues of Control (Vehicle treated), Negative Control (STZ treated), STZ+AC (100 mg/kg, p.o.), STZ+AC (100 mg/kg, p.o.), STZ+AC (100 mg/kg, p.o.) treated rats. It was observed that level of Aβ1-40 and Aβ1-42 in the brain tissue of negative control group (STZ only treated rats) increases significantly (p<0.01) compared to control group of rats. However, this increased level of Aβ1-40 and Aβ1-42 were found to be decreased significantly (p<0.01) in AC treated group compared to AD rats (Negative control group). This decrease in the level of Aβ1-40 and Aβ1-42 plays a role in the decrease in the neuron degeneration.
Figure 3: Effect of *Amorphophallus campanulatus* extract on Aβ1-40 peptide in the brain tissue of STZ treated AD rats. Values are means ± S.E.M. (n=8); @p < 0.01 (vs. Control group), *p<0.05, **p < 0.01 (vs. Negative control group).

Figure 4: Effect of *Amorphophallus campanulatus* extract on Aβ1-42 peptide in the brain tissue of STZ treated AD rats. Values are means ± S.E.M. (n=8); @p < 0.01 (vs. Control group), **p < 0.01 (vs. Negative control group).

Estimation of AchE in brain tissue

Effect of petroleum ether extract of *Amorphophallus campanulatus* on AchE activity in STZ induced AD rats as shown in Fig 2. STZ administration produced significant elevation (P< 0.01) in brain AchE activity compared to control group of rats. However, treatment with *Amorphophallus campanulatus* extract resulted in significant inhibition (P< 0.05) of AchE activity in brain tissue as compared with the negative control group of rats.
Figure 5: Effect of *Amorphophallus campanulatus* extract on AchE in the brain tissue of STZ treated AD rats. Values are means ± S.E.M. (n=8); @p < 0.01 (vs. Control group), **p < 0.01 (vs. Negative control group)

Table 1: Effect of treatment with *Amorphophallus campanulatus* extract on SOD, LPO & CAT in the brain tissue of STZ treated AD rat.

| Sr. No. | Group                  | SOD (Unit/mg protein) | LPO (nmol MDA/ mg protein) | CAT (µmol H₂O₂ consumed/ min/mg protein) |
|---------|------------------------|-----------------------|----------------------------|-----------------------------------------|
| 1       | Control                | 10.5±1.05             | 6.81±0.5                   | 40.5±1.73                               |
| 2       | Negative control       | 3.7±0.6*              | 12.50±1.1*                 | 65.3±2.75*                              |
| 3       | STZ+AC 100 mg/kg       | 5.2±0.4*              | 10.21±1.4*                 | 55.9±2.30*                              |
| 4       | STZ+AC 200 mg/kg       | 8.9±1.8**             | 7.38±0.6**                 | 43.2±1.84**                             |
| 5       | STZ+AC 500 mg/kg       | 9.63±1.4**            | 6.92±0.3**                 | 38.7±1.31**                             |

Values are means ± S.E.M. (n=8); @p < 0.01 (vs. Control group), *p<0.05, **p < 0.01 (vs. Negative control group)

Estimation of oxidative stress parameters

Effect of *Amorphophallus campanulatus* extract on superoxide dismutase, lipid peroxidation, catalase in the brain tissue of STZ treated AD rats were shown in Table 1. STZ induced AD, results in significant (p<0.01) decrease in the SOD and increase (p<0.01) in the LPO and CAT level compared to control group of rats. However treatment with *Amorphophallus campanulatus* extract significantly improved the SOD level in the brain tissues compared to negative control group of rats. LPO and CAT level were found to be significantly decreases (p<0.01) in the brain tissues of *Amorphophallus campanulatus* extract treated group of rats compared to negative control group of rats. Moreover, study result also suggested that these improvement in the level of oxidative stress parameters is dose dependent.

Evaluation of Histopathological study of brain tissue

Histopathological study suggested that there was no plaque of Aβ peptide found in the brain tissue of control group of rats. Observation also suggested that icv administration of STZ develops plaque of Aβ formation in the brain tissues of negative control group. Treatment with AC extract (100 mg/kg, 200 mg/kg & 500 mg/kg) found to be responsible for fairly decrease in the amyloid plaque formation compared to negative control group of rat. Moreover decrease in amyloid plaque formation was found to be in a dose dependent manner (Fig. 6).
Figure 6: Effect of AC extract on the histopathology of STZ induced AD rats brain (40×) a: TS of brain of normal rat: Control group; b: TS of brain of STZ treated rat: Negative control; c: TS of brain of AC (100 mg/kg) treated STZ induced AD rat: STZ+AC (100 mg/kg); d: TS of brain of AC (200 mg/kg) treated STZ induced AD rat: STZ+AC (200 mg/kg); e: TS of brain of AC (500 mg/kg) treated STZ induced AD rat: STZ+AC (500 mg/kg).

Discussion

AD induced by STZ produces progressive deficits in cognitive function in rats which is similar to sporadic kind of AD, as indicated by behavioral tests including passive avoidance paradigm and spatial cognitive deficit in Y-maze task (Bimonte-Nelson et al., 2003; Mohsen and Faezeh, 2010).

Literature suggested that improvement in the % alteration and step through latency behavior in Y maze and single trial passive avoidance test respectively, confirms the improvement in memory and cognitive performance in AD rats (Khalili and Hamzeh, 2010).

Results of present study demonstrated that treatment with AC (100 mg/kg, 200 mg/kg & 500 mg/kg) ameliorates the cognitive function in STZ induced AD rats.

Reported studies reveal that spatial cognitive deficits are characterized by changes at the level of various neurotransmitters and related markers (Sharma & Gupta, 2002; Lannert & Hoyer, 1998).

Cholinergic system is the most severely affected body system in spatial cognitive deficits (Carr et al., 1997) and elevation of the Ach level might be helpful in improve the symptoms of cognitive deficits in Alzheimer's disease (Gasparini et al., 1998). Result of this study suggested that treatment with AC extract reduces the AchE level in the brain tissues of AD rats. Increased level of βA peptide in the brain tissues increases the levels of AchE and on the basis of this it results in neurodegeneration (Cioanca et al., 2013).

Treatment with AC extract found to be reduces the concentration of βA peptide significantly in the brain tissue of STZ induced AD. ICV administration of STZ elevates the oxidative stress and there by alters the cholinergic markers and βA peptide level in the brain tissues of AD rats (Sharma and Gupta, 2002).

AC extracts possesses strong antioxidant properties by improving SOD, LPO and CAT level in the brain tissues of STZ induced AD rats.

Conclusion

The present study concludes that AC possesses neuroprotective effect in STZ induced AD rats. Study also postulates the mechanism of its action as treatment with AC reduces oxidative stress which decreases the concentration of βA peptide in the AD rat. This decreased concentration of βA peptide in the AD rat attenuates the AchE level which increases the concentration of acetylcholine in the brain tissue and thereby it protects the neurodegeneration in STZ induced AD rats.

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