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Original Research Article

Protective Effect of Garlic Extract Against Pentylenetetrazole Induced Seizures

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ABSTRACT

Background and Aims: Previous studies in literature reported that compared with fresh preparations of garlic and aged garlic extract (AGE) contain high amount of antioxidants and protect against cancer, aging and many cardiovascular diseases. The present work investigates effect of AGE against pentylenetetrazole (PTZ)-induced convulsions and brain levels of Gamma amino butyric acid (GABA).

Methods: The least and median convulsive (CD_{50}) doses of PTZ were determined either alone or after pretreatment with AGE. The forebrain levels of the neurotransmitter GABA have been measured in rats given PTZ, alone and after pretreatment with AGE.

Results: The present study showed that AGE pretreatment elicited a dose dependent protection of rats against PTZ-induced seizures. AGE in a dose of 400 mg 60 minutes prior to 60mg/kg PTZ produced 80% decrease in incidence of seizures. AGE pretreatment produced significant elevation of the median convulsive dose (CD50) of PTZ from 47 mg/kg to 55 mg/kg. Results have shown that cellular brain GABA concentrations decreased significantly ($P < 0.05$) in rats given 400 mg/kg AGE 60 minutes prior to 60 mg/kg PTZ compared with control and PTZ-treated groups.

Conclusion: The present work suggests a potential neuroprotective effect of AGE against PTZ-induced seizures that may be explained by increasing brain levels of the major central inhibitory neurotransmitter GABA.

Key Words: Pentylenetetrazole, Seizures, Gamma amino butyric acid (GABA).

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INTRODUCTION

Garlic is considered a component of traditional medicine that has long been used for hypertension[1] and diabetes.[2,3] It is a health protecting food owed to its antioxidant/anti-inflammatory and neuroprotective activities. Aged garlic extracts (AGE), compared with fresh garlic preparations, has no odor, contains higher quantities of antioxidants and protects against cancer, many cardiovascular diseases, stroke, Alzheimer's disease and other aging disorders.[4] To get higher quantities of antioxidants, AGE is prepared by extracting and aging fresh garlic at room temperature for about one and half years in 20 % ethanol. AGE contains flavonoids, allixin, selenium and water-soluble sulfur antioxidant substances with high bioavailability such as S-allylmercaptocysteine (SAMC) and S-allyl cysteine (SAC).[5,6]

Pentylenetetrazole (PTZ) is a prompt and powerful central nervous system (CNS) stimulant. The principal target cells for PTZ are those in the medulla and midbrain. In larger doses its action ascends to the cerebral cortex and descends to the spinal cord.

In experimental animals, threshold convulsive doses of the drug produce motor activity characterized by forelimb and jaw clonus. This convulsion resembles that produced by electrical stimulation of the brain with current of just threshold intensity. With slightly larger doses of PTZ, generalized, asynchronized clonic movements are observed.

This phase is usually superseded by a tonic convulsion; such a convulsion resembles that produced by maximal brain stimulation in that the movements of the limbs consist of flexion followed by extension.[7]

In the past, PTZ was used therapeutically as a respiratory stimulant in emergencies and in the treatment of mental disorders (as a shock therapy), but it is no longer used as its margin of safety is too low. PTZ can be used diagnostically. When given in very small amounts, it will produce seizure in a person with epilepsy. Its wide use is to induce seizures for screening of anticonvulsant drugs.[8]

γ-aminobutyric acid (GABA) is the major neurotransmitter for fast inhibitory synaptic transmission. The GABAergic system is a target for a wide range of drugs including anxiolytics, sedative-hypnotics, general anesthetics, and anticonvulsants.[9]

This study provides an insight on the effect of administration of AGE prior to challenging rats with the known convulsing agent PTZ. In the present work, the least and median convulsive dose (CD50) of PTZ was determined either alone or after pretreatment with AGE. The study also investigates the effect of pretreatment of rats with AGE on the central levels of the major inhibitory neurotransmitter GABA in rats after induction of convulsions by PTZ.

MATERIALS AND METHODS

Chemicals

PTZ (Sigma, USA) was dissolved in distilled water. Liquid suspension of AGE (240 mg AGE/ml) was purchased from Wakunaga, USA and administered intraperitoneally at doses of 200 mg/kg and 400 mg/kg.

Animals

Adult male rats weighing 150-200g were used. The animals were group housed in plastic cages and maintained under standard laboratory conditions with a natural light-dark cycle. Rats were left to acclimatize to the environment for at least a week before the experiments. Food and water were allowed *ad libitum*.

Pentylenetetrazol seizures

3 groups of rats each was comprised of 5 animals.

Treatment schedules

Group A: Was given PTZ i.p in a dose of 30 mg/kg.

Group B: Was given PTZ i.p in a dose of 60 mg/kg.

Group C: Was given PTZ i.p in a dose of 70 mg/kg.

Doses were selected according to Malhotra and Gupta.[10] The animals were observed for 30 minutes after PTZ challenge for ***incidence, latencies*** and ***character*** of seizures.

Determination of CD_{50} of PTZ

Computations of the median convulsive dose and its confidence limits for PTZ were proceeded according to the method of Litchfield and Wilcoxon.[11] Groups of 10 rats were injected with graded doses of PTZ. Percentage incidence of seizures in each group was determined during a period of 30 minutes after drug administration.

Effect of AGE pretreatment on PTZ induced seizures

6 groups of rats each was comprised of 10 animals.

Treatment schedules

Group A: Was given PTZ i.p in a dose of 60 mg/kg.

Group B: Was given AGE i.p in a dose of 200 mg/kg 5 minutes before PTZ challenge.

Group C: Was given AGE i.p in a dose of 400 mg/kg 5 minutes before PTZ challenge.

Group D: Was given AGE i.p in a dose of 200 mg/kg 15 minutes before PTZ challenge.

Group E: Was given AGE i.p in a dose of 400 mg/kg 15 minutes before PTZ challenge.

Group F: Was given AGE i.p in a dose of 400 mg/kg 60 minutes before PTZ challenge.

Animals were observed for 30 minutes after PTZ challenge for incidence of myoclonic jerks and generalized tonic clonic seizures. Dose of PTZ was selected according to results obtained in the previous step, while doses of AGE were selected according to preliminary experiments.

Effect of AGE pretreatment on CD_{50} of PTZ

Groups of 10 rats were injected i.p with graded doses of PTZ 5 minutes after their pretreatment with AGE injected i.p in a dose of 400 mg/kg. Percentage incidence of seizures in each group was determined during a period of 30 minutes after PTZ administration. Computation of the median convulsive dose (CD_{50}) and its confidence limits for PTZ were proceeded according to the method of Litchfield and Wilcoxon.[11]

Measurement of GABA levels in rat forebrain

Animal protocol and rat brain extraction

GABA levels in forebrains of the following groups of rats were measured:

1. Control normal rats weighing 150-200g (negative group) comprised of 5 animals.
2. Positive control group challenged with 60 mg/kg PTZ.
3. Rats given AGE i.p in a dose of 400 mg/kg 5 minutes prior to 60 mg/kg PTZ.
4. Rats given AGE i.p in a dose of 400 mg/kg 15 minutes prior to 60 mg/kg PTZ.
5. Rats given AGE i.p in a dose of 400 mg/kg 60 minutes prior to 60 mg/kg PTZ

According to the method described by Laura and Ognen, rats were decapitated and brains were quickly removed (<90 seconds) rostral to the cerebellum and frozen in liquid nitrogen. Frozen brains were extracted in 3.5 ml cold 12% perchloric acid (PCA) stock solution containing 7.7 mM dichloracetic acid (Sigma) and centrifuged at 3200×g for 15 minutes at 4 °C. The neutral supernatant was centrifuged at 3200×g for 10 minutes at 4 °C. 0.5 g chelating resin (Sigma) was added to the neutral solution which is then filtered and lyophilised. The dried powder was dissolved in neutral 50 mM deuterated phosphate in D2O containing 2 mM isopropanol.[12]

Statistical analysis of the results

CD50 values and analysis of the results obtained in the convulsive tests were calculated by fitting the data by linear regression analysis as described by Litchfield and Wilcoxon (1949). Significance tests of CD50 values of theophylline, alone and after pretreatment with Ginger extract in rats were determined by using 95% confidence limits according to Snedecor.[13] The significance of the differences was determined using the student's t-test. The difference was regarded as significant when $P < 0.05$ and as a highly significant when $P < 0.01$.

RESULTS

Pentylenetetrazole seizures

Group A (PTZ 30 mg/kg): No convulsions were elicited.

Group B (PTZ 60 mg/kg): Three rats showed intermittent clonic convulsions with tail erection. The latencies of incidence of convulsions differed (5 – 10 minutes post-injection). These convulsions continued for about 1 minute. One rat showed intermittent clonic convulsions 5 minutes post-injection followed, within seconds, by generalized tonic convulsions then died. One animal showed no convulsions and survived.

Group C (PTZ 70 mg/kg): Generalized tonic-clonic convulsions occurred in the 5 animals 1 minute after injection of PTZ and within seconds; 3 of them died. So, in view of these observations,

PTZ was used in a dose of 60 mg/kg to elicit seizures (clonic intermittent followed by generalized tonic-clonic). This dose was used in the following steps after treating rats with adenosine and its agonists.

Determination of CD50 of PTZ

The median convulsive dose (CD_{50}) of Pentylenetetrazole injected intraperitoneally into rats was equivalent to 47 (40.52 – 54.52) mg/kg.

Table 1: CD50 and its 95% confidence limits of i.p PTZ

Dose	Convulsed -------	Observed	Expected	Observed -	Contribution to
(mg/kg)	tested	% convulsed	% convulsed	expected	$(Chi)^2$
30	0/10	2	6	4	0.0280
40	3/10	30	30	0	0.0000
50	6/10	60	60	0	0.0000
60	8/10	80	82	2	0.0028
70	10/10	97.7	93	4.7	0.0350
				Total	**0.0658**

Total animals = 50
Number of doses = 5
Animals/doses = 50/5 = 10
$(Chi)^2$ = 0.0658 X 10 = 0.658
Degrees of freedom = 5 – 2 = 3
Tabulated $(Chi)^2$ for n of 3 from table (13) = 7.82
.. $(Chi)^2$ calculated is less than $(Chi)^2$ tabulated
∴ The data are not significantly heterogenous
CD_{84} = 62 mg/kg
CD_{50} = 47 mg/kg
CD_{16} = 35 mg/kg

$$S = \frac{62/47 + 47/35}{2} = \frac{1.32 + 1.34}{2} = \frac{2.66}{2} = 1.33$$

$\overline{N}$ = 30
Exponent = 2.77/ $\sqrt{30}$ = 2.77/5.47 = 0.51
$FCD_{50} = 1.33^{0.51} = 1.16$
CD_{50} X FCD_{50} = 54.52
CD_{50}/FCD_{50} = 40.52
∴ CD_{50} and its 95% confidence limits = 47 (40.52 – 54.52) mg/kg.

Effect of AGE pretreatment on PTZ-induced seizures

Group A (PTZ 60 mg/kg):

Within 3-5 minutes there were myoclonic jerks that continued for about 1 minute. This was followed by generalized clonic convulsions with appearance of tail erection in some ones. Generalized seizures continued for a brief duration (less than 1 minute) and then the animals died.

Group B (AGE 200 mg/kg 5 minutes before PTZ 60 mg/kg): Myoclonic seizures were observed in 8 rats about 20 minutes after injection of PTZ and continued for about 1 minute. In 6 out of the eight rats, seizures became generalized for brief seconds and the animals then survived. Two rats showed irritability without incidence of convulsions.

Group C (AGE 200 mg/kg 15 minutes before PTZ 60 mg/kg): Myoclonic intermittent convulsions happened in 5 rats about 20 minutes after injection of PTZ and within 30 seconds became generalized tonic-clonic seizures and the animals died later.

Group D (AGE 400 mg/kg 5 minutes before PTZ 60 mg/kg): Myoclonic convulsions occurred in 4 rats about 15 minutes after injection of PTZ and continued for about 1 minute then became generalized clonic seizures for 30 seconds and the animals survived.

Group E (AGE 400 mg/kg 15 minutes before PTZ 60 mg/kg): Myoclonic seizures happened in 3 rats about 30 minutes after injection of PTZ and continued for about 30 seconds. One of the previous 3 rats showed generalized convulsions with tail erection for about 10 seconds and survived. The remainder rats showed no abnormality.

Group F (AGE 400 mg/kg 60 minutes before PTZ 60 mg/kg): Myoclonic seizures were observed in two rats 30 minutes after PTZ challenge and lasted for about 1 minute. Then it became generalized clonic in one of the two rats. The generalized convulsions continued for less than one minute and the animals convulsed animals survived.

Effect of AGE pretreatment on CD50 of PTZ

The median convulsive dose of PTZ injected intraperitoneally into rats 5 minutes after AGE pretreatment was equivalent to 55 (46.6 – 64.9) mg/kg.

Table 2: CD50 and its 95% confidence limits of i.p. PTZ injected following AGE pretreatment.

Dose	Convulsed -------	Observed	Expected	Observed -	Contribution to
(mg/kg)	tested	% convulsed	% convulsed	expected	$(Chi)^2$
30	0/10	1.6	5	3.4	0.0250
40	2/10	20	20	0	0.0000
50	4/10	40	40	0	0.0000
60	6/10	60	60	0	0.0000
70	8/10	80	74	6	0.0200
80	10/10	95.3	85	10.3	0.0800
				Total	**0.125**

Total animals = 60
Number of doses = 6
Animals/doses = 60/6 = 10
$(Chi)^2$ = 0.125 X 10 = 0.25
Degrees of freedom = 6 – 2 = 4
Tabulated $(Chi)^2$ for n of 4 from table (13) = 9.49
.. $(Chi)^2$ calculated is less than $(Chi)^2$ tabulated
∴ The data are not significantly heterogenous
CD_{84} = 79
CD_{50} = 55
CD_{16} = 38

$$S = \frac{79/55 + 55/38}{2} = \frac{1.44 + 1.45}{2} = \frac{2.89}{2} = 1.44$$

$\overline{N}$ = 40
Exponent = 2.77/ $\sqrt{40}$ = 2.77/6.32 = 0.44
$FCD_{50} = 1.44^{0.44} = 1.18$
CD_{50} X FCD_{50} = 64.9
CD_{50}/FCD_{50} = 46.6
∴ CD_{50} and its 95% confidence limits = 55 (46.6 – 64.9) mg/kg

Glutamate and GABA levels in rat forebrain

Cellular brain GABA levels increased significantly (P < 0.05) in rats given AGE i.p in doses of 400mg/kg 5, 15 and 60 minutes prior to 60mg/kg PTZ compared with negative and PTZ -treated groups.

Table 3: GABA Brain levels

Group	GABA level
Negative control	2.18 ± 0.04
Positive control (PTZ 60mg/kg)	1.65 ± 0.02*
AGE i.p in a dose of 400 mg/kg 5 minutes prior to 60mg/kg PTZ	2.52 ± 0.01*#
AGE i.p in a dose of 400 mg/kg 15 minutes prior to 60mg/kg PTZ	2.48 ± 0.02*#
AGE in a dose of 400 mg/kg60 minutes prior to 60mg/kg PTZ	2.64 ± 0.01*#

Values represent the mean concentrations (mM) with ± SEM.
**P* <0.05 versus negative control.
# *P* <0.05 versus PTZ.

DISCUSSION

Garlic and its preparations have been long used to maintain good health and as a component of traditional medicine for treatment and protection against many cardiovascular and metabolic disorders such as hypertension, atherosclerosis, hyperlipidemia, thromboembolism, degenerative diseases as dementia, cancer and diabetes mellitus.[14] Aged garlic extract is available with known dose concentration over the counter in a suitable form for intake.

Contrary to fresh garlic; AGE is highly acceptable, has no odor with minimal gastrointestinal troubles. It is prepared by storing macerated sliced fresh garlic at room temperature for 20 months. Aging of garlic increases the content of water-soluble compounds and decreases the oil-soluble sulfur compounds that have the unpleasant odour.[15] AGE contains high amount of S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) in addition to stable lipid-soluble allyl sulfides, flavonoids and saponins.[16]

Pentylenetetrazole is a known CNS stimulant widely used for screening of anticonvulsant drugs. Stimulant effects of PTZ may also be due to the capacity to reduce the impact of GABA on chloride ion conductance at concentrations that do not alter resting neuronal membrane conductance in the absence of GABA.[17] In addition, PTZ appears to interact with binding sites for picrotoxin and to block the enhanced binding of GABA and benzodiazepines that is produced by hypnotic barbiturates.[18] While direct excitatory effects have not been excluded, it would appear that a major action of PTZ may be to reduce GABA-ergic inhibition, thereby enhancing CNS excitability.

Our study demonstrated that AGE, in a dose of 400 mg/kg if given 5 minutes before PTZ in a dose of 60 mg/kg, was partially effective in protection against PTZ-induced seizures as evidenced by decreased number of convulsing rats and an increased latency before convulsions.

It was also observed that AGE (400 mg/kg) pretreatment produced significant elevation of the median convulsive dose (CD50) of PTZ in rats from 47 mg/kg to 55 mg/kg. The anticonvulsant effect of age observed is this work may be explained by the high content of antioxidants. This is in agreement with Gupta et al., 2002 have demonstrated that resveratrol, a polyphenolic compound with potent antioxidant activity, when administered 20 minutes prior to convulsive challenge with PTZ (60 mg/kg i.p), it dose-dependently reduced the percentage of generalized tonic-clonic convulsions.[19] More recently, Orozco-Ibarra and his colleagues reported that AGE and its main constituent S-allylcysteine (SAC) are natural antioxidants that have protective effects against cerebral ischemia.[20]

As aforementioned, the enhancing CNS excitability effect of PTZ may be owed to reduction of GABA-ergic inhibition, the present work illustrated how administrations of AGE prior to induction of convulsions by PTZ; alter the cellular brain levels of the major inhibitory neurotransmitter GABA. Results have shown significant increase in GABA levels (P < 0.05) in rats given AGE 5 minutes prior to 60 mg/kg PTZ compared with control group and PTZ-treated group.

The present study is in agreement with other studies in literature with regards to effect of garlic extract on GABAergic neurotransmission. Dinesh and Vaibhav, 2008 have been reported that garlic extract showed antidepressant-like activity through interaction with adrenergic, dopaminergic, serotonergic and GABAergic systems.[21] Another more recent study by Neeraj and Dinesh, 2016 mentioned showed that garlic extract possesses an anxiolytic effect and increases GABA brain levels in mice.[22]

CONCLUSION

Based on the results obtained in the present study, it seems that AGE may have a notable protective effect against PTZ-induced convulsions that may be owed to its antioxidant activities or enhancement of the inhibitory GABA neurotransmission.

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