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Caffeine Ameliorating Effect on Anxiety and Depression in an Aluminum Chloride-induced Alzheimer's Disease Rat Model

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ABSTRACT

This study was conducted to evaluate the long-term ameliorating effect of caffeine in adult male Wistar rats (Aluminum Chloride- $AlCl_3$ induced AD Model). A total number 64 Wistar adult male rats, with average weight of 200 g were used and randomly assigned into 4 groups (n= 16), 4 replicates each. In control group (C), they had plain water ; in group two, Alzheimer-induced group (AD) rats had ad libitum supplied with $AlCl_3$ in drinking water at 200 mg/kg/day ; in group three : Caffeine group (CF), the rats had Caffeine (80 mg/kg body weight) in drinking water ; and in group (4) (CF-AD, the rats were exposed to ad libitum supply of $AlCl_3$ and caffeine alternatively in the drinking water. The treatment was continuous for 16 weeks using in a day after day regime of administration of $AlCl_3$ and Caffeine and plain water. The measuring parameters were behavioral parameters using open field test and forced swim test. The determined oxidative stress parameters included Malondialdehyde and Glutathione. The obtained results revealed that there were significant effects of caffeine on the measured behavioral and biochemical variables. The treated rats were differently influenced by caffeine. The observations revealed that caffeine had a significant anxiolytic effect on the measured behavioral profile in the open field, and antidepressant influence in forced swim test. Furthermore, the measured biomarkers proved the antioxidant effect achieved by the long-term caffeine administration in the studied AD model. It was concluded that caffeine improves the behavioral profile of anxiety and depression in Alzheimer-induced rat models, and has an ameliorating effect. These results supported the new approaches of using caffeine in therapeutic potentials for Alzheimer management.

Key words: *Alzheimer's Disease, Anxiety, Depression, Antioxidant, Oxidative Stress, Caffeine, Rat Model, Aluminum Chloride.*

INTRODUCTION

Alzheimer's disease (AD) is the most common form of chronic diseases among the elderly. It is a progressive neurodegenerative disease that is characterized by gradual memory loss, and reduction of cognition related functions [1]. With a high old age population in the advanced world, and increasing the shelf life expectancy in the developing world, AD is a big problem around the world [2].

There are various mechanisms of neuronal degeneration in AD having been proposed, which include texture of free radicals [3], oxidative stress [4], mitochondrial dysfunction [5], inflammatory processes [6], genetic factors [7], environmental effect factors [8], and apoptosis [7]. These agents could interact and grow up with each other in a vicious cycle of toxicity, leading to neuronal dysfunction, cell dysfunction, and finally cell death.

Alzheimer's disease (AD) has caused damages to the brain cells that sustain mood, so feelings of depression or anxiety are common in this situation. Therefore, some researchers have suggested that the early depression in AD is

related to the disease's primary brain-altering effects, which may interfere the neurotransmitters that affect the mood, even at the early step of its development [9].

There has been no effective treatment for Alzheimer's disease. A lot of research efforts have been focused on developing new drugs from nutritional supplements which have multifunctional properties, especially with promising antioxidant effects [10].

Coffee, tea, cocoa, energy drinks, chocolate and many other products contain Caffeine (1, 3, 7-trimethylxanthine) which is the most widely used denoting drug that affect a person's mental state [11]. Although it is mainly caffeine-related anxiogenesis that has been described, there are many reports of the opposite result with low doses, namely anxiolysis [12].

Doses in the range were reported by some authors to be anxiety-inducing, although there is also evidence of anxiolysis in rodents usually with low doses [13]. Moreover, [14] reported that in rats, the acute and long-term exposure to caffeine can reverse the anxiogenic effects of unpredictable chronic stress.

The present study aimed to examine the ameliorating effect of habitual administration of caffeine on anxiety and depression behavioral profile of Aluminum Chloride Induced AD rat models.

MATERIALS AND METHODS

Animals and housing :

Total number of 64 Wistar adult male rats, approximately of 200 ± 50g weight were procured from Laboratory Animals Breeding Unit, Department of Animal behavior and Management, Faculty of Veterinary Medicine, Cairo University, and used in this study.

Animals were maintained on a 12-h light/dark cycle at a room temperature of 20-22°c and 60% humidity. They were fed ad libitum standard laboratory pellets (Al-fagr Pvt. Ltd., Egypt) and water was administrated daily throughout the study. The animal housing and handling were in accordance with Committee for Control and Supervision of Experiments on Animals guidelines. The prior permission for the study was obtained from Institutional Animal Ethics Committee (IAACUC).

Chemicals :

Aluminum chlorides ($AlCl_3.6H_2O_2$) in form of white powder, as well as caffeine, that have been used in the present study were purchased from Alpha Chemika Company, India, Egypt supplied. Both chemicals were freshly prepared at the day of administration.

Experimental design :

The animals were randomly assigned into 4 groups, (n=16 rats), 4 replicates each as follows :

Group one [Control (C)] : rats were given plain water.

Group two [Alzheimer-induced (AD)] : rats exposed to ad libitum supply of $AlCl_3$ in drinking water at 200 mg/kg/ day after day [15].

Group three [Caffeine group (CF)] : rats were given Caffeine (80 mg/kg body weight/ day after day in drinking water,

Group four [Ameliorated group (CF-AD)] : rats were exposed to ad libitum supply of $AlCl_3$ and caffeine alternatively in the drinking water.

The long-term treatment was continuous for 16 weeks.

Measuring Parameters

Behavioural parameters :

All behavioral tests were conducted by the same personnel throughout the study, including 16 weeks of administration. All behaviors were measured at 9.00 A. M. and 15.00 P. M. Hand operated counters and stop watches were used to score animals' behavior. Behavioral tests were separated by at least 24 h from each other, and executed in the following sequence [16].

Open Field Test :

The open field has been long introduced as an appropriate test for measuring situational anxiety in rodents [17]. The open field used in this study was a square wooden arena of (90 x 90 x 25cm). The wood of the apparatus was covered with a plastic laminate (Formica), which does not allow the absorption of fluids (urine of rats). 36 small squares of (15 x 15cm) were obtained through dividing the floor by black lines. 70% ethyl alcohol was used to clean the open field maze for each rat to avoid odor cues.

The rats were taken to the test room in their home cages, and tested for 3 minutes once at a time. The rats were handled by the base of their tails at all times. They were taken from their home cages, and placed randomly into one of the four corners of the open field facing the center. During the test duration of three minutes, the assessment of anxiety including measuring the time spent for freezing (immobility), exploratory behaviours in the form of ambulation (horizontal locomotion) and rearing (vertical activity) as well as non-exploratory measures including only vegetative behaviours in the form of number of faecal boluses (defecation) and the number of times of urination, was done [18]. These parameters have been used to indicate the level of anxiety [19].

Forced Swim Test :

The rats were examined in the forced swim test as formerly explained by [20]. They were placed in cylindrical container (50 x 20 cm) filled with 30 cm of 22oC water. Generally, in this test, the water level does not let the rat rest on its tail or run away the cylinder by climbing out. Each rat was placed in the water for 6 min. The time spent floating (represented immobility) was scored during the last 3 min. The time spent immobile was considered as an index of depression-like behavior in rodents [21].

Oxidative stress parameters :

To complete all behavioral assessments, six rats per treatment were sacrificed by cervical decapitation under ether anesthesia for whole brain tissue extraction. Brain tissue specimens were exposed and removed carefully from each rat, chilled for 15 min, and thereafter homogenization was done. Fifty hundred micro liters of tissue was homogenate in ice cold 10 % potassium chloride for homogenization, then followed by centrifugation in cooling centrifuge at 4 0C for 20 min at 5000 rpm, and the supernatant was obtained. The samples were analyzed on an Agilent HP 1100 series HPLC apparatus (USA), according to [22], and performed at National Organization for Drug Control and Research, Giza, Egypt.

Determination of Malondialdehyde (MDA)

MDA standard was firstly prepared according to [23]. Then the estimation of total MDA in the brain samples were performed (nmol/g).

Determination of (GSH, GSSG)

The thiols compounds of oxidized and reduced glutathione were detected by HPLC using the method of [24]. Glutathione (oxidized and reduced) reference standard was purchased from Sigma Chemical Co. Dissolved in 75% methanol in stock 1mg/ml and diluted before application to HPLC.

Statistical analysis :

Data analysis for all variables were carried out by means of analyses of variance (ANOVA) to judge the ameliorating effect of administration of caffeine to rats as well as session factor for behavioral tests using the general linear models procedure in SPSS® statistical software (SPSS, 2016). After confirmation of significant effects in the overall ANOVA, the data for different groups were compared using post hoc Tukey HSD test. For all tests, the criterion for statistical significance was $p<0.05$. The results were reported as mean ± SEM.

RESULTS:

Open field test :

Anxiety state of the animals was assessed in the open field test as shown in Table 1. Aluminum Chloride administrated rats showed higher levels of anxiety-related behaviors than others in the control group. That was indicated by increased time spent freezing ($F_{(1, 60)} = 9.79$; $p = 0.00$), lower vertical activity (numbers of rearing(; ($F_{(1, 60)} = 15.16$; $p = 0.00$) , less horizontal exploration ($F_{(1, 60)} = 9.40$; $p = 0.03$) elevated defecation scores ($F_{(1, 60)} = 17.86$; $p = 0.00$)as well as urination scores ($F_{(1, 60)} = 12.19$; $p = 0.00$).

On the other hand, significance ($p < 0.05$) increased in care, and the mean covered distance in the open field quiz for caffeine-AD treated persons were compared with the AD control group. Furthermore, the administration of caffeine along with $AlCl_3$ (CF-AD group) significantly reduced freezing time ($F_{(1, 60)} = 4.60$; $p = 0.04$),fecal boluses ($F_{(1, 60)} = 11.54$; $p = 0.00$) and urination scores ($F_{(1, 60)} = 8.27$; $p = 0.01$). Also, rats given caffeine alone displayed a marked significant ($p < 0.01$) decrease in freezing time ($F_{(1, 60)} = 6.61$; $p = 0.01$) and vegetation scores; ($F_{(1, 60)} = 4.22$; $p = 0.04$) for defecation score, and ($F_{(1, 60)} = 6.60$; $p = 0.01$) for urination score. Furthermore, caffeine treatment alone (CF group) had significant influence on horizontal activity (numbers of crossed squares); ($F_{(1, 60)} = 6.03$; $p = 0.02$)as well as vertical activity ($F_{(1, 60)} = 15.90$; $p = 0.00$).

Table 1. Effect of $AlCl_3$ and its amelioration by caffeine on anxiety measurements in open field test in rats.

Gp Parameters	(C) Group	(AD) Group	(CF) Group	(CF-AD) Group
Freezing time(s)	2.31 ±1.96[a]	20.06 ±26.17[b]	0.88 ±1.45[ac]	4.18 ±5.84[acd]
Horizontal activity	52.06 ±27.99[a]	23.75 ±12.17[b]	57.12 ±19.96[ac]	48.38 ±31.74[acd]
Vertical activity	10.00 ±5.76[a]	3.00 ±2.00[b]	13.44 ±6.46[ac]	10.13 ±5.77ac
Defecation scores	1.31 ±1.20[a]	4.19 ±1.64[b]	1.63 ±1.71[ac]	1.94 ±1.44[adc]
Urination scores	0.94 ±1.12[a]	2.88 ±1.20[b]	1.19 ±1.11[ac]	1.38±1.41[ac]

(C) Group : Animals received plain water without any treatment and served as a control.
(AD) Group : Animals received 200 mg/kg $AlCl_3$ in drinking water.
(CF) Group : Animals received 80 mg/kg caffeine in drinking water.
(CF-AD) Group : Animals received caffeine 80 mg/kg along with $AlCl_3$ in drinking water.
[a-d]Values within row with unlike superscripts differ significantly (p<0.05), according to ANOVA.
Values represent Mean ±SE of 16 animals per treatment (n=16).

Force swimming test :

Table 2 shows the stability time during physical power in swimming test. This measure was increased significantly in AD rats ($F_{(1, 60)} = 35.70$; $p = 0.00$). Also, (CF group) showed significant influence in decreasing immobility time ($F_{(1, 60)} = 33.77$; $p = 0.00$). While decrease in immobility time was noted in the caffeine ameliorated AD rats which was not significantly different when compared to AD group.

Oxidative stress parameters (MDA, GSH, GSSG) :

MDA activity as well as GSH and GSSG contents in the brain tissues of rats are presented in table 3. MDA activity significantly increased ($p < 0.05$) in $AlCl_3$ exposed rats (AD) compared to their control. Significant reduction of this alleviation was recorded when caffeine was concomitantly administered (CF-AD). However, caffeine alone had no significance on MDA measure compared to the control group.

The Level of GSH was significantly higher ($p < 0.05$) while GSSG formation was reduced significantly in case of AD rats. This reduction in GSH was enhanced by administration of caffeine, but without significant difference, while the elevation of GSSG formation was significantly diminished compared to AD rats. Caffeine treatment alone had no significant influence on brain tissue for both GSH and GSSG levels.

Table 2. Effect of $AlCl_3$ and its amelioration by caffeine on the behavior of rats during the forced swim test.

Gp Parameters	(C) Group	(AD) Group	(CF) Group	(CF-AD) Group
Immobility time (s)	96.56±18.45[a]	129.56±15.74[b]	62.25±27.60[c]	97.50±27.16[a]

(C) Group : Animals received plain water without any treatment and served as a control.
(AD) Group : Animals received 200 mg/kg $AlCl_3$ in drinking water.
(CF) Group : Animals received 80 mg/kg caffeine in drinking water.
(CF-AD) Group : Animals received caffeine 80 mg/kg along with $AlCl_3$ in drinking water.
a-dValues within row with unlike superscripts differ significantly (p<0.05), according to ANOVA.
Values represent Mean±SEM of 16 animals per treatment (n=16).

Table 3. The Effect $AlCl_3$ and its amelioration by caffeine on the oxidative stress markers (MDA, GSH, GSSG) of brain Hippocampus in male rats.

Gp Parameters	(C) Group	(AD) Group	(CF) Group	(CF-AD) Group
MDA nmol/g	22.60 ± 1.63[a]	40.65 ± 1.59[b]	22.34 ± 1.04[a]	24.87 ± 1.10[c]
GSH µmol/g	10.21 ± 0.93[a]	6.51 ± 0.36[b]	10.04 ± 0.35[a]	7.55 ± 0.43[b]
GSSG µmol/g	0.30 ± 0.02[a]	0.64 ± 0.06[b]	0.31 ± 0.03[a]	0.37 ± 0.03[c]

(C) Group : Animals received plain water without any treatment and served as a control.
(AD) Group : Animals received 200 mg/kg $AlCl_3$ in drinking water.
(CF) Group : Animals received 80 mg/kg caffeine in drinking water.
(CF-AD) Group : Animals received caffeine 80 mg/kg along with $AlCl_3$ in drinking water.
a-cValues within row with unlike superscripts differ significantly (p<0.05), according to ANOVA.
Values represent Mean±SEM of 16 animals per treatment (n=6).

DISCUSSION:

Neurotoxicity of Aluminum chronic exposure is involved in the initiation and progression of various cognitive and non-cognitive disorders like Alzheimer's disease [25]. In this study AD model was induced by chronic aluminum exposure, and the behavioral changes and the possible ameliorating effect of caffeine using behavioral and biochemical tests were investigated.

The open field measures elucidated the anxiety related behaviors in adult male Wistar rats. AD rats were found to have typical anxiety behavioral profile that was significantly noted in their lower horizontal and vertical exploration, higher freezing time and vegetation scores, compared to their counterparts from the control group. These results were in an agreement with those of [26] that showed an increase in the anxiety like behavior in AD rat models.

AD rats treated with caffeine significantly showed higher levels of locomotion,as a measure of less anxiety, indicated by increased numbers of crossing squares. Also, caffeine treated group showed lower freezing time accompanied with higher level of rearing, compared to AD rats. Defecation and urination frequency was enhanced in caffeine treated group, and according to [27, 28], the fecal boli and urination are sensitive measures for anxiety state of animals.

In contrast to the results of this study, the anxiogenic effect of caffeine has been documented in a substantial literature [29, 30]. Caffeine has been reported to elicit a dose-dependent, subjective feeling of anxiety, even at low doses [12, 31]. The stimulation of anxiety in response to caffeine administration was explained as a result of increased levels of lactate in the brain [32, 33]. This discrepancy in results, and the anxiolytic effect of caffeine on the behavioral changes might be attributable to the different administered doses, and the caffeine's complex mechanisms as a neuroprotective agent.

The antioxidant and chelating properties of Aluminum have also been involved, as well as modulation of cell-signaling and cell survival pathways. In agreement with the findings of this study, increased activity of animals was experienced after administration of low or moderate doses of caffeine [34, 35]. Moreover, [36] have observed an increase in both home cage and open field activity in rats following low doses of caffeine.

The force swim test has been used to measure behavioural despair and depression in rodents [37]. In this examination, data of forced swimtask revealed that AD group exhibited higher immobility; that is considered asindex of depression-like behavior as a react to increased levels of stress reaction. Further support obtained something from previous study where elevated aluminum in response to chronic stress was associated with increasing manifestations of depression [38].

On the other hand, it was noticed that there was a significant decrease in the immobility mean rank of (CF-AD) rats as compared with AD group. This in turn reflected the success of putative antidepressant effect of caffeine in reversing the depressive like behavior resulted in the group of AD model. Furthermore, the findings revealed that the (CF group) has recorded the lowest significant mean rank for immobility time among the other groups including the control one. The position of caffeine in alleviation of depression response has been previously studied [39].

Regarding the oxidative stress biomarkers, AD group showed obvious changes in MDA, and reduced and oxidized glutathione as they were significantly different from the control group. These results confirmed the oxidative hypothesis in Alzheimer's disease etiology that had been reported by [40]. Comparing to (CF-AD) group, the ameliorating effect of caffeine administration demonstrated high antioxidant effect on the measured parameters, indicated by significantly reduction in MDA level as well as GSSG, whereas GSH elevated with no significance. These findings are in accordance with [41] that reported beneficial outcomes after caffeine administration including a decrease in MDA, associated with an increase in GSH to GSSG ratio.

CONCLUSION:

According to the results obtained, this study potently suggests the relevance of caffeine in inducing ameliorating effects especially on behaviors related to anxiety and depression. Furthermore, it had potent antioxidant activity that could help in alleviating both cognitive and non-cognitive profile of AD. Further investigations on natural antioxidants are needed in order to find out the most suitable approaches for management of such disease.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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