Anxiolytic activity of Angiotensin-Receptor-Blocker in Experimental Models of Anxiety in Mice

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Abstract: The present study aimed to explore the role of Angiotensin-Receptor-Blocker in the management of anxiety. Male Swiss albino mice of age 6-8 weeks and weight 25-30 g were used in the present study. Candesartan (Angiotensin receptor blocker) was administered in two doses (1 and 2 mg/kg; *i.p.*) to mice for 14 successive days regularly. Anxiety was induced in mice by two different methods: (i) exposing the mice to immobilization stress for a period of 6 h daily for 7 consecutive days; (ii) administration of caffeine (25 mg/kg; *i.p.*) daily for 7 days. Elevated Zero Maze and Open Field Apparatus were used to evaluate the level of anxiety in different groups. After behavioral evaluation, the animals were sacrificed and their brains were used for estimation TBARS, GSH and Nitrite levels in the brain. Administration of Candesartan (1 and 2 mg/kg; *i.p*) for 14 successive days significantly ($p<0.05$) reduced anxiety due to immobilization stress and caffeine induced anxiety. Candesartan (1and 2 mg/kg; *i.p*) treated mice showed an increase ($p<0.05$) in GSH levels while a decrease ($p<0.05$) in TBARS and nitrite levels in brain. Thus, candesartan may prove to be a useful remedy for the management of anxiety owing to its neuroprotective and antioxidant activity.

Keywords: Angiotensin, Candesartan, anxiety, immobilization stress, caffeine

1. INTRODUCTION

Anxiety is an emotional state commonly caused by the perception of real or perceived danger which threatens the security of an individual characterized by various behavioral components. The physical symptoms of anxiety

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disorders may include restlessness, fatigue, mental tension, irritability, sleep disturbances, poor stress management, abdominal distress, chest discomfort, palpitations, tachycardia, shortness of breath, trembling, blushing, diarrhea, sweating, nausea and chills etc. It is the most prominent stress- related psychiatric disorder which can produce uncomfortable and potentially debilitating psychological feeling of distress. Everyone experiences a certain amount of nervousness and apprehension when encountered with a stressful situation. This is an adaptive response and transient in nature. However, when it becomes excessive it may come under the category of a disorder (Saavedra *et al*., 2005). According to recent reports of National institute of Mental Health (NIMH, USA) lifetime prevalence rate of anxiety affected adults was 18.1%, out of which 22.8% were severely affected; while lifetime prevalence rate for 13 to 18 years old was 25.1%, out of which 5.9% were severely affected. Demographic representations for lifetime prevalence show that women are 60% more likely than men to experience an anxiety disorder.

Higher centers in brain (such as amygdala, hypothalamus and locus coeruleus) and abnormal function in neurotransmitter systems (including norepinephrine, gamma amino butyric acid and serotonin) are involved in the provocation of anxiety (Gabry *et al*., 2003, Nutt, 2000). Dysregulation of the brain noradrenergic system may be a factor in determining vulnerability to stress related pathology, as stress activates norepinephrine release in a number of stress related limbic regions, such as the central and medial amygdala, lateral bed nucleus of the stria terminalis, medial prefrontal cortex, and lateral septum (Morilak *et al*., 2005). Further, serotonin (5-HT) has been shown to be involved in a number of pathophysiological processes such as depression, hypertension and anxiety (Jenkins, 2008). Furthermore, evidence suggests that behavioral indices of anxiety are associated with the promotion of dopamine activity within mesolimbocortical and mesolimbic sites. Stimuli like mild to moderate stressors activate central dopamine activity, and this effect can be prevented by the administration of anxiolytic agents (Ferrer *et al*., 2001). One of the major defining neurobiological features of anxiety is hyperactivity of hypothalamo-pituitary-adrenal axis (HPA) with subsequent elevation in glucocorticoid levels (released by the adrenal cortex) in response to stress. (Burroughs and French, 2007). Therefore, any stressful event leads to provocation of anxiety by altering the release pattern of neurotransmitter in the brain (Tanaka *et al*., 2000). Recently, attention has been focused on the role of a brain regulatory peptide, Angiotensin II. It elicits most of its biological actions by binding to specific membrane-bound AT_1 receptors on target cells to activate multiple intracellular transduction pathways (Yin *et al*., 2003). The brain and peripheral angiotensin II increases during stress, activating brain AT_1 receptors,

which causes stress induced hormone secretion, including CRH, ACTH, corticosteroids and vasopressin and in turn stimulates the central sympathetic activity (Valdez and Koob., 2003). Angiotensin II receptor blockers are compounds with a good margin of safety and are widely used in the treatment of hypertension. Their anti-inflammatory and vascular protective effects contribute to reduce renal and cardiovascular failure (Saavedra *et al*., 2011). Long term peripheral administration of angiotensin receptor blocker (candesartan) blocks not only peripheral but also brain AT_1 receptors, preventing the hormonal and sympathoadrenal response to stress. Inhibition of brain AT_1 receptor activity may be neuroprotective by reducing exaggerated stress responses and anxiety, preventing stress induced gastric ulcerations, decreased vulnerability to ischemia and stroke, reversing chronic cerebrovascular inflammation and reduces acute inflammatory responses produced by bacterial endotoxin (Benicky *et al*., 2009). These effects may protect neurons from injury and contribute to increased life span. Inhibition of brain AT_1 receptors in humans is also reported to reduce the incidence of stroke, improving cognition and decrease the progression of Alzheimer's disease (Saavedra *et al*., 2011).

Anxiolytic effects of the angiotensin II AT_1 receptor antagonist losartan were studied in the elevated plus maze and light/dark test in different mouse strains (Gard *et al*., 2001). Blockade of AT_1 receptors offers a novel and safe therapeutic approach for the treatment of illnesses of increasing prevalence and socioeconomic impact, such as anxiety disorders and other neurodegenerative diseases of the brain. However, the literature reports regarding the role of angiotensin receptor blocker is limited. Therefore, the present study was undertaken to explore the potential of candesartan (an angiotensin receptor blocker) in the management of anxiety using stress induced and caffeine induced anxiety models.

2. MATERIALS & METHODS

2.1 Drugs & Chemicals

Candesartan and alprazolam were purchased from Sigma Aldrich (China). Caffeine and thiobarbituric acid, 5,5-Dithiobis (2-nitrobenzoic acid) were procured from Himedia laboratories, Mumbai. Reduced glutathione from SRL, Mumbai. All the other chemicals were of analytical grade.

2.2 Animals

Male Swiss mice weighing between 22-30 g were procured from Disease free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. The experimental protocols were approved by the Institutional Animals Ethics Committee. Animals were housed and maintained

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under standard laboratory conditions with controlled temperature ($23 \pm 2°C$), humidity ($40 \pm 10\%$) and 12 h light and dark cycles each. The animals were fed with standard rodent pellet diet (Ashirwad Industries, Mohali) and water *ad libitum*. The experiments were carried out between 09.00 and 17.00 h. The laboratory animals were maintained as per the guidelines of CPCSEA, Ministry of Environment and Forests, Government of India.

2.3 Induction of Anxiety

Anxiety was induced in animals experimentally by following two methods:

(a) Immobilization Stress: Animals were subjected to immobilization stress of 6 hours daily for 7 consecutive days to both stressed and drug treated groups. Each mouse was gently handled and immobilized individually with their ventral body facing the platform in 50 ml centrifuge tubes with two holes at both the ends so that the animal can properly respire and their tails were kept outside the tubes through rear hole and secured with adhesive tape to the surface so that the animals cannot move at all. The experiments were conducted at fixed time of the day through-out the observation (Sur and Bhattacharya, 1997 & Dhir *et al*., 2006).

(b) Caffeine induced anxiety: Administration of Caffeine (25 mg/kg; i.p.) to mouse for 7 days daily induces anxiety (Oh ki-wan *et al*., 2010).

2.4 Experimental Design

The animals were divided into different groups (n=6). Candesartan was administered daily by intraperitoneal injection to mice for 14 successive days at two doses i.e. 1 and 2 mg/kg. The doses of candesartan were selected on the basis of literature reports (Sanchez-Lemus *et al*., 2012). In immobilization stress model, the animals were subjected to 6 hours of immobilization for 7 successive days, starting from 8th day of candesartan administration upto 14th day. In caffeine-induced anxiety model, caffeine (25 mg/kg; *i.p.*) was administered for 7 successive days starting from 8th day of candesartan administration upto 14th day. On 15th day, the animals were subjected to behavioural assessment using Elevated Zero Maze test and Open Field test. Alprazolam served as standard drug to compare the putative outcomes of candesartan treatment and was administered intraperitoneally for 14 successive days to mice at a dose of 0.25 mg/kg (Nunez *et al.,* 2011). After behavioral evaluation, the animals were sacrificed and brains were used for estimations of thiobarbituric acid reactive substances (TBARS; by method of Ohkawa *et al.,* 1979), reduced glutathione (GSH; by method of Ellman *et al*., 1959) and Nitrite (by method of Green *et al*., 1982) levels. The results were statistically analyzed by one-way ANOVA followed by Tukey's test.

2.5 Elevated Zero Maze Test

The elevated zero maze (EZM) is a sensitive behavioral test that reveals animals neophobia or anxiety and can be used to unveil antineophobic and anxiolytic actions of drugs. This maze is an elevated (40 cm) black, annular having outer diameter of 45 cm and inner diameter of 30 cm. The runway ring where the mouse can explore is of 6 cm width, which is divided into 4 quadrants, 2 opposing "open" quadrants without walls and 2 opposing "closed" quadrants having 12 cm high walls. The open quadrants have a ridge of 2-3mm to prevent the mouse to fall off. The walls have thickness of 0.75 cm. Animals were individually placed in closed arm facing towards the open arm and the following parameters were noted for a period of five minutes (Shepherd *et al*., 1994; Braun *et al*., 2011).

a) Latency to enter the open arm (LEO): The time gap between the first entry of animal in open arm after placing it in the closed arm. Enhancement of LEO as compared to control group indicates induction of anxiety and vice-versa.

b) Time spent in open arm (TSO): It is the time spent by the animal in open arm out of total allotted 5 min. Reduction of TSO indicates induction of anxiety and vice-versa.

c) Number of entries in the open arm (NEO): It is the frequency of entry of animal in the open arm. Higher the frequency of entry in open arm lower is the level of anxiety.

2.6 Open Field test

This behavioral model is based on the induction of anxiety state such as ambulation or freezing by exposing animals to a highly novel field environment. An open field apparatus consists of a circular arena (wall height 27 cm; diameter 84 cm) with 25 houses. Animals were placed in circular open arena of apparatus. When animal moves from one segment to another, one ambulation (simple stereotypy) was recorded. Similarly when the animal stands on its hind limbs, one rearing (complex stereotypy) was recorded. Anxiolytic drug action was reflected by increased central and peripheral ambulation and rearing with decreased fecal boli, grooming and freezing time, in comparison to normal control groups (Kulkarni & Dandiya, 1974).

3. RESULTS

3.1 Effect of candesartan on LEO in elevated zero maze:

Mice exposed to immobilization stress for 6 h for seven consecutive days showed enhancement in ($p<0.05$) LEO in stress control group as compared to

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normal control mice. However, mice pretreated with candesartan (1 or 2 mg/kg; *i.p.*) for 14 days prevented the rise in LEO significantly ($p<0.05$) as compared to stress control group. Alprazolam (0.25 mg/kg; *i.p.*) pretreatment for 14 days also reduced ($p<0.05$) the latency when compared with stress control group. Administration of caffeine (25 mg/kg; *i.p.*) to mice caused increase in LEO ($p<0.05$) in caffeine control group as compared to normal control. Pretreatment of mice with candesartan for 14 days reduced ($p<0.05$) the LEO as compared to caffeine control group. Alprazolam pretreatment for 14 days also reduced ($p<0.05$) the latency when compared with caffeine control group (Figure 1).

3.2 Effect of candesartan on NEO in elevated zero maze

Exposure of mice to immobilization stress for 6 h daily for 7 successive days markedly reduced ($p<0.05$) NEO in stress control group as compared to normal control mice. Pretreatment with candesartan (1 or 2 mg/kg; *i.p.*) for 14 days increased the NEO significantly ($p<0.05$) as compared to stress control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the NED as compared with stress control group. Animals treated with caffeine (25 mg/kg; *i.p.*) showed decreased ($p<0.05$) NEO in caffeine control group as compared to normal control mice. Pretreatment with candesartan for 14 days increased NEO significantly ($p<0.05$) as compared to caffeine control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the number of entries when compared with caffeine control group (Figure 2).
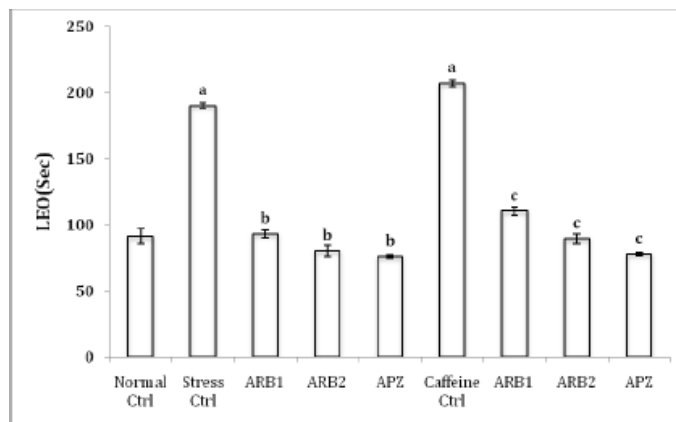

Figure 1: Effect of Candesartan on latency to enter the open arm (LEO) in elevated zero maze.

Values are expressed as mean ± S.E.M. a-$p<0.05$ vs normal control; b- $p<0.05$ vs stress control; c- $p<0.05$ vs caffeine control. ARB1= Candesartan 1mg/kg, ARB2 = Candesartan 2 mg/kg and APZ = Alprazolam 0.25 mg/kg
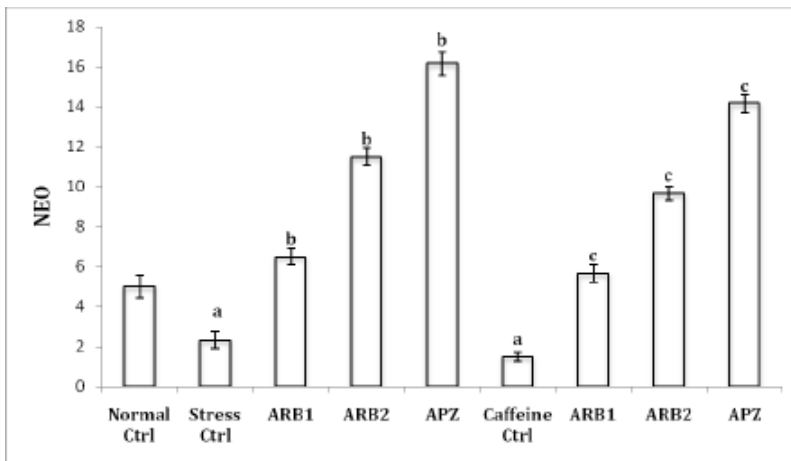
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Figure 2: Effect of Candesartan on Number of entries in the open arm (NEO) in elevated zero maze.

Values are expressed as mean ± S.E.M. a-$p<0.05$ vs normal control; b- $p<0.05$ vs stress control; c- $p<0.05$ vs caffeine control. ARB1= Candesartan 1mg/kg, ARB2 = Candesartan 2 mg/kg and APZ = Alprazolam 0.25 mg/kg.
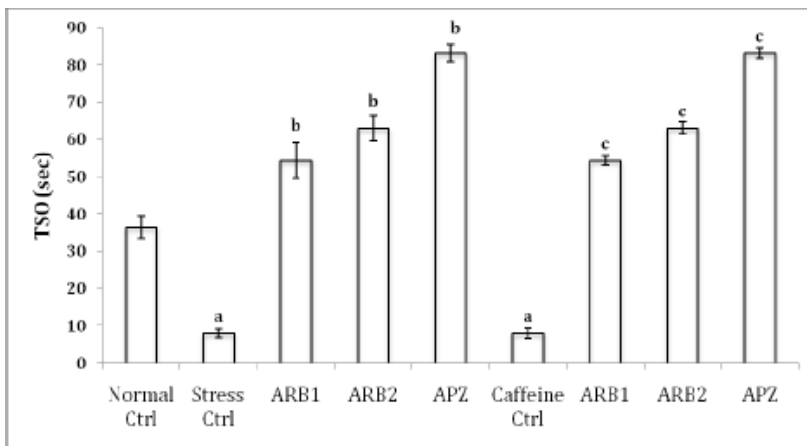

Figure 3: Effect of Candesartan on time spent in the open arm (TSO) in elevated zero maze.

Values are expressed as mean ± S.E.M. a-$p<0.05$ vs normal control; b- $p<0.05$ vs stress control; c- $p<0.05$ vs caffeine control. ARB1= Candesartan 1mg/kg, ARB2 = Candesartan 2 mg/kg and APZ = Alprazolam 0.25 mg/kg.

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3.3 Effect of candesartan on TSO in elevated zero maze

Exposure to immobilization stress showed a decrease ($p<0.05$) in TSO in stress control group as compared to normal control mice. Pretreatment with candesartan (1 & 2 mg/kg; *i.p.*) for 14 days increased the TSO significantly ($p<0.05$) as compared to stress control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the TSO when compared with stress control group. Animals treated with caffeine (25 mg/kg; *i.p.*) showed a decrease ($p<0.05$) in TSO in caffeine control group as compared to normal control. Pretreatment with candesartan for 14 days increased the TSO significantly ($p<0.05$) as compared to caffeine control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the TSO when compared with caffeine control group (Figure 3).

3.4 Effect of candesartan on ambulation behavior of mice in open field

Exposure to immobilization stress showed a decrease ($p<0.05$) in ambulation in stress control group as compared to normal control mice. Pretreatment with candesartan for 14 days increased the ambulation significantly ($p<0.05$) as compared to stress control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the ambulation when compared with stress control group. Animals treated with caffeine (25 mg/kg; *i.p.*) showed decreased ($p<0.05$) ambulation in caffeine control group as compared to normal control. Pretreatment with candesartan for 14 days increased the ambulation significantly ($p<0.05$) as compared to caffeine control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the ambulation when compared with caffeine control group (Table 1).

3.5 Effect of candesartan on rearing behavior of mice in open field

Exposure to immobilization stress showed a decrease ($p<0.05$) in rearing in stress control group as compared to normal control mice. Pretreatment with candesartan (1 & 2 mg/kg; *i.p.*) for 14 days increased the rearing significantly ($p<0.05$) as compared to stress control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the rearing when compared with stress control group. Animals treated with caffeine (25 mg/kg; *i.p.*) showed decreased ($p<0.05$) rearing in caffeine control group as compared to normal control. Pretreatment with candesartan (1 & 2 mg/kg; *i.p.*) for 14 days increased the rearing significantly ($p<0.05$) as compared to caffeine control group. Alprazolam pretreatment for 14 days also increased ($p<0.05$) the rearing when compared with caffeine control group (Table 1).

Table 1: Effect of Candesartan on ambulation and rearing score using open field test.

Group	Ambulation score	Rearing score
Normal control	75.16 ± 7.46	5.5 ± 0.42
Stress control	59.83 ± 2.37[a]	3.0 ± 0.36[a]
Stress + ARB1	103.55 ± 1.78[b]	113.66 ± 0.55[b]
Stress + ARB2	110.68 ± 2.09[b]	14.16 ± 0.7[b]
Stress + Alprazolam	139 ± 4.68[b]	21.66 ± 0.98[b]
Caffeine control	50.16 ± 3.17[a]	3.16 ± 0.47[a]
Caffeine + ARB1	97.5 ± 1.97[c]	12.16 ± 0.79[c]
Caffeine + ARB2	102.5 ± 2.81[c]	12.33 ± 0.8[c]
Caffeine + Alprazolam	145.5 ± 2.07[c]	22.16 ± 1.16[c]

Values are expressed as mean ± S.E.M. a-$p<0.05$ vs normal control; b- $p<0.05$ vs stress control; c- $p<0.05$ vs caffeine control. ARB1= Candesartan 1mg/kg, ARB2 = Candesartan 2 mg/kg and APZ = Alprazolam 0.25 mg/kg.

Table 2: Effect of Candesartan on biochemical parameters.

Group	TBARS	GSH	Nitrite
Normal control	0.3 ± 0.007	2.35 ± 0.13	5.33 ± 0.25
Stress control	0.73 ± 0.008[a]	1.23 ± 0.4[a]	11.44 ± 0.1[a]
Stress + ARB1	0.61 ± 0.007[b]	1.58 ± 0.1[b]	8.25 ± 0.07[b]
Stress + ARB2	0.52 ± 0.007[b]	1.71 ± 0.02[b]	7.52 ± 0.15[b]
Stress + Alprazolam	0.42 ± 0.007[b]	1.89 ± 0.01[b]	7.18 ± 0.08[b]
Caffeine control	0.74± 0.007[a]	1.4 ± 0.07[a]	11.47 ± 0.4[a]
Caffeine + ARB1	0.63± 0.006[c]	1.7 ± 0.03[c]	8.42 ± 0.07[c]
Caffeine + ARB2	0.54± 0.007[c]	1.75 ± 0.02[c]	7.67 ± 0.3[c]
Caffeine + Alprazolam	0.43± 0.007[c]	1.89 ± 0.04[c]	7.34 ± 0.34[c]

Values are expressed as mean ± S.E.M. a- $p<0.05$ vs normal control; b- $p<0.05$ vs stress control; c- $p<0.05$ vs caffeine control. ARB1= Candesartan 1mg/kg, ARB2 = Candesartan 2 mg/kg and APZ = Alprazolam 0.25 mg/kg.

3.6 Effect of candesartan on biochemical parameters

The animals exposed to immobilization stress showed higher brain TBARS and nitrite level ($p<0.05$) as compared to normal control mice, however GSH

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levels were reduced. Pretreatment with candesartan (1 & 2 mg/kg; *i.p.*) for 14 days not prevented the stress-induced rise in brain TBARS as well as nitrite level ($p<0.05$) but also prevented the reduction of GSH levels as compared to stress control group. Alprazolam pretreatment for 14 days also ($p<0.05$) prevented the stress-induced oxidative damage as expected. The animals treated with caffeine also showed higher brain TBARS and nitrite levels ($p<0.05$) as compared to normal control mice. Pretreatment with candesartan (1 & 2 mg/kg; *i.p.*) for 14 days prevented the caffeine-induced rise in brain TBARS / nitrite level ($p<0.05$) and fall in GSH levels as compared to caffeine control group (Table 2)

4. DISCUSSION

The present study investigates the probable role of angiotensin receptor blocker (candesartan) in relieving anxiety in rodents. The result of elevated zero maze describes a possible anxiolytic effect of candesartan as shown by the increased number of entries arm and enhancement in total time spent in open arm in immobilization as well as caffeine-induced anxiety groups. The same pattern of results was observed in open field test. Immobilization stress is a useful tool to induce anxiety in rodents by immobilizing the animal through physical means. This model combines emotional stress (escape reaction) and physiological stress (muscle work), resulting in both restricted mobility and aggression (Saavedra *et al*., 2012; Dhir *et al*., 2006). Caffeine exerts dose related effect on rodents, showing locomotor stimulation at lower dose while anxiogenic like effects at higher dose. At higher dose caffeine increases GABA$_A$ receptor expression in cerebro-cortical membranes (Jain *et al*., 2005; Oh Ki-wan *et al.,* 2010). In the present study, a significant rise in anxiety was observed in immobilization stress-induced and caffeine-induced anxiety groups as compared to control animals. As a stress hormone, norepinephrine affects the parts of the brain such as amygdala which plays a key role in the regulation of stress (Saavedra *et al*., 2011*).* Gelband *et al*., 1997 states that neuronal AT$_1$ receptors are localized on the plasma membrane of neuronal cell soma and the hypothalamus and brainstem nuclei contain high concentrations of angiotensin receptors and their stimulation results in profound effects on catecholamine turnover. Many types of physical stress such as immobilization stress or restraint stress increases brain angiotensin II formation and upregulates the AT$_1$ receptors within the blood brain barrier specifically in HPA axis which contributes to the stimulation of stress pathways in the brain (Quirin *et al*., 2008). This stimulation of brain angiotensin II activity ultimately results in higher HPA axis activation, enhanced responses to stress, and increased

anxiety. AT_1 receptor activation alters norepinephrine uptake, synthesis, and release from hypothalamus brainstem neuronal cells (Saavedra & Benicky, 2007). Therefore, it is suggested that norepinephrine has implications in stress induced anxiety. Caffeine at higher dose increases the rate of synthesis and turnover of norepinephrine. However, the mechanism by which caffeine activates the noradrenaline neurons is not known (Nutt, 1990). Therefore, it is quite possible that the observed anxiolytic effect of candesartan may be by virtue of affecting norepinephrine levels through AT_1 receptors. Furthermore, it is also proposed by previous reports that administration of candesartan may relieve anxiety by modulating norepinephrine levels (Saavedra *et al*; 2005 & 2011).

In the present study, we report the antioxidant property of candesartan as demonstrated by reduction in brain TBARS as well as Nitrite levels and enhancement in reduced GSH levels after administration for 14 days to stressed animals. Stress causes enhancement of oxidative damage and results in enhancement of lipid peroxidation and reduced antioxidant defense (Li *et al*., 2013). In the present study, animals subjected to both immobilization stress and caffeine induced anxiety exhibited an increase in brain TBARS levels and decrease in GSH levels. Stress causes oxidative injury and malondialdehyde (MDA) is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation product. MDA readily participates in nucleophilic addition reaction with 2-thiobarbituric acid (TBA), generating thiobarbituric acid reactive substances, which is an indicator of increased lipid peroxidation (Janero, 1990). However, glutathione is a ubiquitous thiol-containing tripeptide that is produced in all organs and it is implicated in the cellular defense against xenobiotics and naturally occurring deleterious compounds, such as free radicals and hydroperoxides. Glutathione not only protects cell membranes from oxidative damage, but also helps to maintain the sulfhydryl groups of many proteins in the reduced form, a requirement for their normal function (Trachootham *et al*., 2007).

It is worthwhile to mention here that angiotensin receptor blockers are mainstay of antihypertensive therapy. The rise in blood pressure is one of the symptoms and hallmark of anxiety associated disorder. The possibility that the decrease in blood pressure by administration of candesartan may be contributing to relieve the anxiety in mice, cannot be ruled out. However, the dose of candesartan required for eliciting antihypertensive activity and anxiolytic activity may be an intercept. In spite of all this discussion, further investigations are very much warranted to consolidate the potential of angiotensin receptor blockers in the management of anxiety.

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REFERENCES

[1] Benicky, J., Sanchez-Lemus, E., Pavel, J., Saavedra, J.M. (2009). Anti-inflammatory effects of angiotensin receptor blockers in the brain and the periphery. Cellular and Molecular Neurobiology, 29, 781-792. doi: 10.1007/s10571-009-9368-4.
http://dx.doi.org/10.1007/s10571-009-9368-4

[2] Braun, A.A., Skelton, M.R., Vorhees, C.V., Williams, M.T. (2011). Comparison of the elevated plus and elevated zero mazes in treated and untreated male Sprague-Dawley rats: Effects of anxiolytic and anxiogenic agents. Pharmacology, Biochemistry & Behavior, 97, 406–415.
http://dx.doi.org/10.1016/j.pbb.2010.09.013

[3] Burroughs, S., French, D. (2007). Depression and anxiety: Role of mitochondria. Current Anaesthesia and Critical Care, 18, 34-41. http://dx.doi.org/10.1016/j.cacc.2007.01.007

[4] Dhir, A., Padi, S.S.V., Naidu, P.S., Kulkarni, S.K. (2006). Protective effect of naproxen (non-selective COX-inhibitor) or rofecoxib (selective COX-2 inhibitor) on immobilization stress-induced alterations in mice. European Journal of Pharmacology, 535, 192-198.
http://dx.doi.org/10.1016/j.ejphar.2006.01.064

[5] Ellman, G.L. (1959). Tissue sulfhydryl groups. Archives of Biophysics and Biochemistry, 82, 70-77. http://dx.doi.org/10.1016/0003-9861(59)90090-6

[6] Ferrer, D.B., Reymann, J.M., Tribut, O., Allain, H., Vasar, E., Bourin, M. (2001). Role of dopaminergic and serotonergic systems on behavioral stimulatory effects of low-dose alprazolam and lorazepam. European Neuropsychopharmacology, 11, 41-50.
http://dx.doi.org/10.1016/S0924-977X(00)00137-1

[7] Gabry, K.E., Chrousos, G., Gold, P.W. (2003). The Hypothalamo-Pituitary-Adrenal (HPA) axis: A major mediator of adapytive responses to stress. Neuroimmune Biology, 3, 379-414.
http://dx.doi.org/10.1016/S1567-7443(03)80055-8

[8] Gard, R.P., Haig, S.J., Cambursano, P.T., Warrington, C.A. (2001). Strain differences in the anxiolytic effects of losartan in the mouse. Pharmacology, Biochemistry and Behavior, 69, 34-40. http://dx.doi.org/10.1016/S0091-3057(01)00491-9

[9] Gelband, C.H., Sumners, C., Lu, D., Raizada, M.K. (1997). Angiotensin receptors and norepinephrine neuromodulation: implications of functional coupling. Regulatory Peptides, 72, 139-145. http://dx.doi.org/10.1016/S0167-0115(97)01050-1

[10] Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R. (1982). Analysis of Nitrate, Nitrite and [15N] Nitrate in biological fluids. Analytical Biochemistry, 126, 131-138. http://dx.doi.org/10.1016/0003-2697(82)90118-X

[11] Jain, N.S., Hirani, K., Chopde, C.T. (2005). Reversal of caffeine-induced anxiety by neurosteroid 3-alpha-hydroxy-5-alpha-pregnane-20-one in rats. Neuropharmacology, 48, 627-638.
http://dx.doi.org/10.1016/j.neuropharm.2004.11.016

[12] Janero, D.R. (1990). Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free radical biology & Medicine, 9, 515-540.
http://dx.doi.org/10.1016/0891-5849(90)90131-2

[13] Jenkins, T.A. (2008). Effect of angiotensin-related antihypertensives on brain neurotransmitter levels in rats. Neuroscience letters, 444, 186-189. http://dx.doi.org/10.1016/j.neulet.2008.08.021

[14] Kulkarni S.K., Dandiya, P.C. (1974). A preliminary report on the action of imipramine and antiparkinsonian agents on the open field behavior of rats. Japan Journal of Pharmacology, 24, 809-10. http://dx.doi.org/10.1254/jjp.24.809

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of Angiotensin-
Receptor-Blocker
in Experimental
Models of Anxiety
in Mice

[15] Liu, N., Wang, L.H., Guo, L.L., Wang, G.Q., Zhou, X.P., Jiang, Y., Shang, J., Murao, K., Chen, J.W., Fu, W.Q., Zhang, G.X. (2013). Chronic restraint stress inhibits hair growth via substance P mediated by reactive oxygen species in mice. PLoS One. 8, e61574. doi: 10.1371/journal. pone.0061574. http://dx.doi.org/10.1371/journal.pone.0061574

[16] Morilak, D.A., Barrera, G., Echevarria, D.J., Garcia, A.S., Hernandez, A., Ma, S., Petre, C.O. (2005). Role of brain norepinephrine in the behavioral response to stress. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 29, 1214-1224. http://dx.doi.org/10.1016/j.pnpbp.2005.08.007

[17] Nunez, M.J., Novio, S., Amigo, G., Garabal, M.F. (2011). The antioxidant potential of alprazolam on the redox status of peripheral blood leukocytes in restraint-stressed mice. Life Sciences, 89, 650-654. http://dx.doi.org/10.1016/j.lfs.2011.07.027

[18] Nutt, D.J. (1990). The pharmacology of human anxiety. Pharmacology & Therapeutics 47: 233-66. http://dx.doi.org/10.1016/0163-7258(90)90089-K

[19] Nutt, D.J. (2000). The psychobiology of posttraumatic stress disorder. Journal of Clinical Psychiatry, 61S, 24-29.

[20] Oh Ki-Wan, Park, K.S., Oh, J.H., Yoo, H.S., Lee, Y.M., Lee, M.K., Hong, J.T. (2010). (-)-Epigallocatechin-3-o-gallate (EGCG) reverses caffeine induced anxiogenic-like effects. Neuoscience Letters, 481, 131-134. http://dx.doi.org/10.1016/j.neulet.2010.06.072

[21] Ohkawa, H., Ohishi, N., Yagi, K. (1979). Assay for lipid peroxides in animal tissues by Thiobarbituric acid reaction. Analytical Biochemistry, 95, 351-358. http://dx.doi.org/10.1016/0003-2697(79)90738-3

[22] Quirin, M., Pruessner, J.S., Kuhl, J. (2008). HPA system regulation and adult attachment anxiety: Individual differences in reactive and awakening cortisol. Psychoneuroendocrinology, 33, 581-590. http://dx.doi.org/10.1016/j.psyneuen.2008.01.013

[23] Saavedra, J.M., Ando, H., Armando, I., Baiardi, G., Bregonzio, C., Juorio, A., Macova, M. (2005). Anti-stress and anti-anxiety effects of centrally acting angiotensin II AT1 receptor antagonists. Regulatory Peptides, 128, 227-238. http://dx.doi.org/10.1016/j.regpep.2004.12.015

[24] Saavedra, J.M., Benicky, J. (2007). Brain and peripheral angiotensin II play a major role in stress. Stress, 10, 185-193. http://dx.doi.org/10.1080/10253890701350735

[25] Saavedra, J.M., Lemus, E.S., Benicky, J. (2011). Blockade of brain angiotensin II AT1 receptors ameliorates stress, anxiety, brain inflammation and ischemia: Therapeutic implications. Psychoneuroendocinology, 36,1-18. http://dx.doi.org/10.1016/j.psyneuen.2010.10.001

[26] Sánchez-Lemus, E., Honda, M., Saavedra, J.M. (2012). Angiotensin II AT1 receptor blocker candesartan prevents the fast up-regulation of cerebrocortical benzodiazepine-1 receptors induced by acute inflammatory and restraint stress. Behaviour Brain Research, 232, 84–92. http://dx.doi.org/10.1016/j.bbr.2012.03.041

[27] Shepherd, J.K., Grewal, S.S., Fletcher, A., Bill, D.J., Dourish, C.T. (1994). Behavioural and pharmacological characterisation of the elevated "zero-maze" as an animal model of anxiety. Psychopharmacology, 11, 656-64.

[28] Sur, T. K., Bhattacharyya, D. (1997). The effect of panax ginseng and diazepam on brain and hypothalamic 5-hydroxytryptamine during stress. Indian Journal of Pharmacology, 29, 318–321.

[29] Tanaka, M,, Yoshida, M., Emoto, H., Ishii, H. (2000). Noradrenaline systems in the hypothalamus, amygdala and locus coeruleus are involved in the provocation of anxiety: basic studies. European Journal of Pharmacology, 405, 397-406. http://dx.doi.org/10.1016/S0014-2999(00)00569-0

Hasan, S.R.
Sinha, M.
Bansal, N.

[30] Trachootham, D., Lu, W., Ogasawara, M.A., Valle, N.R., Huang, P. Redox Regulation of Cell Survival. (2007). Antioxidant Redox Signal. 10, 1343–1374. http://dx.doi.org/10.1089/ars.2007.1957

[31] Valdez, G.R., Koob, G.F. (2004). Allostasis and dysregulation of corticotropin-releasing factor and neuropeptide Y systems: implications for the development of alcoholism. Pharmacology Biochemistry and Behavior, 79, 671-689. http://dx.doi.org/10.1016/j.pbb.2004.09.020

[32] Yin, G., Yan, C., Berk, B.C. (2003). Angiotensin II signaling pathways mediated by tyrosine kinases. The International Journal of Biochemistry & Cell Biology, 35, 780-783. http://dx.doi.org/10.1016/S1357-2725(02)00300-X