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RESEARCH PAPER

EFFECT OF ANGIOTENSIN RECEPTOR BLOCKER (CANDESARTAN) ON CHRONIC FATIGUE SYNDROME IN MICE

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The present study was aimed to explore the role of AT_1 receptor blocker (candesartan) in the management of chronic fatigue syndrome. Swiss albino mice (either sex; 6-8 weeks and 20-30 g) were used in this study. Chronic fatigue was induced in mice by two different methods: (i) exposing the mice to forced swimming daily for 10 min for 21 days; (ii) administration of single dose of LPS (1 mg/kg; *i.p.*) to mice followed by forced swimming daily for 10 min for 21 successive days. Candesartan was administered daily in 2 doses (1 and 2 mg/kg; *i.p.*) to mice for 21 successive days. Behavioural assessment such as immobility time, elevated plus maze (for memory), elevated zero maze (for anxiety), open field (for ambulation) and tail-immersion test (for stress induced hyperalgesia) were used to evaluate the induction of fatigue. After behavioural evaluation, blood glucose, blood cortisol, brain TBARS and GSH levels were also estimated. Administration of candesartan significantly ($p<0.05$) reduced the immobility time of mice as compared to control group. Further, administration of candesartan significantly ($p<0.05$) prevented memory impairment, exerted anxiolytic activity and reduced hyper sensitivity to pain of mice. Candesartan treated mice showed significant ($p<0.05$) reduction in blood cortisol levels as compared to FS control group however, enhanced the cortisol levels compared to LPS control group. Candesartan treated mice showed a significant ($p<0.05$) increase in GSH and decrease in brain TBARS. Thus, candesartan may prove to be a useful remedy for the management of chronic fatigue syndrome.

Key words: Angiotensin-receptor blocker, Candesartan, Chronic fatigue syndrome, Oxidative stress.

INTRODUCTION

Chronic fatigue syndrome (or CFS) is a disease characterized by prolonged fatigue for a period of six months or longer that is not improved by taking rest and may be exacerbated by physical or mental activity (Wessely, 2001). CFS results from a variety of stress conditions, such as prenatal stress, early life stress, physical stress, mental stress, emotional stress and stress caused by bacterial endotoxin. The illness occurs most often in people aged 40-59 and it occurs more frequently in women than in men. The condition affects an estimated 42 persons out of every 10,000 (Jason *et al* 2005). The symptoms of CFS may include physical and mental exertion, cognitive impairment, disturbed sleep patterns, musculoskeletal pain, sore throat and headaches (White, 2010). The causes of CFS are immune system abnormalities and chronic immune activation, dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, brain abnormalities, sleep disorders, emotional stress (comprising host aspects) and infections, for example, various microbial infections (Epstein-Barr virus, enteroviruses, parvovirus B19, *Coxiella burnetii* and *Chlamydia pneumoniae*),

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vaccinations and exposure to organophosphate chemicals and other toxins (comprising environmental aspects) (Sanders and Korf, 2008). Although there is no specific treatment available for CFS but anti-depressant agents, corticosteroids and anticholinergic agents are utilized for symptomatic relief. It has been reported that during an immune response (such as bacterial infection), certain cytokines like interleukin (IL)-1, IL-6 and TNF-alpha can signal the brain, which triggers activation of both the central nervous system and hypothalamic-pituitary-adrenal axis (HPA axis) (Gupta *et al* 2009). The activation of HPA-axis causes the release of cortisol through CRH and ACTH. Furthermore, the release of cortisol stimulates the juxtaglomerular apparatus (JGA) of kidney which results in release of renin in systemic circulation and in turn rise in angiotensin-II levels (Quirin *et al* 2008). Increased circulating angiotensin II, coupled with increased AT_1 receptor expression in anterior pituitary, adrenal zona glomerulosa and adrenal medulla contribute to the enhanced ACTH, corticosterone, aldosterone and catecholamine formation and release. Excessive brain AT_1 receptor activity is associated with exaggerated sympathetic and hormonal response to stress, vulnerability to cerebrovascular ischemia and brain inflammation, processes leading to neuronal injury (Allen *et al* 2000). Therefore, the HPA axis acts as a major mediator of adaptive response to immune response and stress. The biological as well as behavioural features of CFS may be linked to endocrine dysfunction of the HPA axis. Many types of experimental stress is induced by immobilization, restraint, cold-restraint, isolation, forced swim, infection with bacterial endotoxin and inflammation, increase brain angiotensin II formation and upregulate brain AT_1 receptor transcription and expression, in particular in the hypothalamic paraventricular nucleus and subfornical organ (Bregonzio *et al* 2008).

Inhibition of brain AT_1 receptor activity may be achieved with the use of orally administered ARBs, of tested efficacy in the treatment of cardiovascular disease and a good margin of safety (Baiardi *et al* 2004). In animal models, inhibition of brain AT_1 receptor activity with systemically administered Angiotensin II receptor blockers is neuroprotective; it reduces exaggerated stress responses and anxiety, prevents stress-induced anxiety, fatigue and gastric ulcerations, decreases vulnerability to ischemia and stroke, reverses chronic cerebrovascular inflammation, and reduces acute inflammatory responses produced by bacterial endotoxin (Saavedra *et al* 2011). The anti-ischemic, anti-stress and anti-inflammatory effects of ARBs indicate that these compounds may be considered as contributors to the therapy of a wide range of conditions, including mood disorders and neurodegenerative diseases of the brain (Anderson, 2010).

However, no sufficient studies have been carried out to explore the role of ARBs in chronic fatigue syndrome, to best of our knowledge. Therefore, the present study aimed at investigating the role of AT_1 receptor blocker in experimental models of Chronic Fatigue Syndrome in mice.

MATERIALS AND METHODS

Drugs and chemicals

Candesartan (Sigma Aldrich, Mumbai) was dissolved in 0.1 N Na_2CO_3, diluted in isotonic saline at a final pH of 7.0-8.0. Dexamethasone Sodium Phosphate (Decdan®) injection (Wockhardt limited, Mumbai), Lipopolysaccharide from Sigma Aldrich (Mumbai), Thiobarbituric acid and 5,5-Dithiobis (2-nitrobenzoic acid) from Hi-media laboratories (Mumbai). All other chemicals used were of analytical grade.

Animals

The experimental protocol of this research project has been approved by Institutional Animal Ethics Committee of the institute (Approval No. ASCB/IAEC/06/13/82). Swiss albino mice (either sex) weighing 20-30 g and aged 6-8 weeks were procured from Disease free small animal house, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. The animals were kept in quarantine section till monitoring of health status of received animals and subsequently transferred to the housing area.

The animals were acclimatized for seven days to the housing conditions of Central Animal House Facility prior to experiments. Animals were housed and maintained under standard laboratory conditions with controlled temperature (23 ± 2°C), humidity (40 ± 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.

Induction of chronic fatigue syndrome

Forced swimming (FS)

The animals were forced to swim individually in glass jar (25 cm × 12 cm × 25 cm) containing 15 cm deep water at room temperature (23 ± 2°C) for a period of 10 min daily and this is repeated for 21 successive days.

After an initial period of vigorous activity each animal assumed atypical immobile posture. The mice were considered to be immobile when they ceased to struggle and made minimal limb movements to keep their head above the water level. The immobility period was noted for a period of 6 min in a total period of 10 min on alternate days for 21 successive days (Kulkarni, 1999).

Lipopolysaccharide (LPS) induced model in mice followed by forced swimming

Mice challenged with single dose of LPS (1mg/kg; *i.p.*), followed by equivalent volume of isotonic saline (vehicle of drug) administered for 21 successive days (Gupta *et al* 2009) with the daily exposure of forced swimming for 10 min.

Experimental design

The animals were divided into different groups (n=6). Candesartan was administered daily by intra-peritoneal injection to mice for 21 successive days at two doses *i.e.* 1 and 2 mg/kg; *i.p.* The doses of candesartan were selected on the basis of literature reports (Sanchez-Lemus *et al* 2012).

In forced swimming (FS), the animals were forced to swim individually at room temperature for 21 successive days, starting from 1st day of candesartan administration upto 21 days. In LPS induced model, a single dose of LPS (1 mg/kg; *i.p.*) followed by equivalent volume of isotonic saline administered for 21 successive days along with forced swim session.

On 22nd day animals were subjected to behavioural assessment using Elevated plus maze test (EPM), Elevated zero maze test (EZM), Open field apparatus and Tail-immersion test. After behavioural evaluation, the animals were sacrificed and brains were isolated for TBARS and GSH estimations.

Groups of animals for chronic fatigue syndrome

Animals were divided into 10 groups (n=6)

Group I: (Normal)

Normal saline was administered for 21 days.

Group II: (ARB2 per se)

Candesartan (2 mg/kg; i.p.) was administered for 21 successive days.

Group III: (Forced swimming i.e. FS)

Mice were forced to swim individually for 10 min daily for 21 successive days.

Group IV: (ARB1)

Candesartan (1 mg/kg; *i.p.*) was administered to mice daily for 21 successive days 30 min before the mice were forced to swim.

Group V: (ARB2)

Candesartan (2 mg/kg; *i.p.*) was administered to mice daily for 21 successive days 30 min before the mice were forced to swim.

Group VI: (Dexamethasone)

Dexamethasone (0.5 mg/kg; *i.p.*) was administered daily prior the mice were forced to swim for 21 successive days.

Group VII: (ARB2 + Dexamethasone)

Dexamethasone (0.5 mg/kg; *i.p.*) and candesartan (2 mg/kg; *i.p.*) were administered to mice daily for 21 successive days each with a gap of 30 min. The animals were forced to swim 60 min after the administration of dexamethasone and candesartan.

Group VIII: (Lipopolysaccharide induced)

Mice were administered with a single dose of LPS (1 mg/kg; *i.p.*) followed by equivalent volume of isotonic saline (vehicle of drug) and were forced to swim daily for 21 successive days.

Group IX: (LPS + ARB2)

Single dose of LPS (1 mg/kg; *i.p.*) and candesartan were administered to mice for 21 successive days each with a gap of 30 min. The animals were forced to swim 60 min after the administration of LPS and candesartan.

Group X: (LPS + ARB2 + Dexamethasone)

Single dose of LPS (1 mg/kg; *i.p.*), dexamethasone and candesartan were administered to mice for 21 successive days each with a gap of 30 min. The animals were forced to swim 1 h 30 min after the administration of LPS, dexamethasone and candesartan.

Behavioural assessment

Elevated plus maze test

Cognitive behaviour was noted by using elevated plus-maze learning task (Kulkarni and Reddy, 1996). Transfer latency (TL) is the time taken by the mouse to move from open arm to enclosed arm with its four paws. Reduction in TL (Transfer Latency) indicates improvement in memory and vice versa. The elevated plus maze consisted of two open arms (16 × 5 cm) and two closed arms (16 × 5 × 12 cm) with an open roof. The maze was elevated to a height of 25 cm from the floor.

The animal was placed individually at the end of either of the open arms and the initial transfer latency was noted on the first day. If the animal did not enter an enclosed arm within 90 s, it was gently pushed into the enclosed arm and the transfer latency was assigned as 90 s. To become acquainted with maze the animal is kept for 20 s after reaching the closed arm and then returned to its home cage. Retention of the learned task was assessed 24 h after 1st day trial.

Elevated zero maze test

The elevated zero maze is a sensitive behavioural test that reveals animals neophobia or anxiety and can be used to unveil antineophobic and anxiolytic actions of drugs (Shepherd *et al* 1994). 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 2-3 mm 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.

Latency to enter the open arm (LEO)

Latency is the time gap between the first entry of animal in open arm after placing it in the closed arm and signifies the behaviour of animal. In the condition of anxiety LEO increases significantly as compared to normal animals.

Total time each animal spent in open arm (TEO)

Average time spent in open arm by the animal indicates the anxiety level. Lower the anxiety level of animal more is the TEO.

Total number of entries in the open arm (NEO)

The frequency of entry of animal in the open arm indicates the behaviour of animal. Higher the frequency of NEO lower is the level of anxiety.

Open field test

This behavioural 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 an animal moves from one segment to another, one ambulation (simple stereotypy) was recorded. Drug action was reflected by increased central and peripheral ambulation in comparison to normal control groups (Srinivasan *et al* 2003).

Tail-immersion test

Tail-immersion test was used to assess hyperalgesic effect in mice. Each mouse was placed individually in restrainer leaving the tail hanging out freely. The terminal 1 cm part of the tail was immersed in a water bath maintained at (52.5 ± 0.5°C).

The withdrawal latency was defined as the time for the animal to withdraw its tail from water. A cut-off time of 15 s was used to prevent damage to the tail (Dhir *et al* 2005). Hyperalgesic response was significantly decreased compared to that of unstressed animals measured on day 22.

Biochemical estimations

***Brain Thiobarbituric acid reactive substances* (Ohkawa *et al* 1979)**

Brain TBARS levels were estimating the absorbance at 532 nm using UV/ Visible spectrophotometer (Shimadzu 1700, Singapore).

***GSH in brain* (Ellman, 1959)**

Brain reduced glutathione levels were estimated by taking absorbance of reaction product at 412 nm using UV/Visible spectrophotometer.

Blood glucose estimations (ACCU-CHEK® Inform Glucose Meter)

Blood sample was collected by retro-orbital method. The second drop of blood was touched to the curved edge of the yellow target area on the Accu-Chek test strip and reading was obtained.

Statistical analysis

All the results were expressed as Mean ± SEM.

The data of all groups were analyzed by one-way ANOVA followed by Tukey's test using software GraphPad InStat (GraphPad Software Inc., USA). A value of P<0.05 was considered to be significant.

RESULTS

Effect of candesartan on immobility period (IP) in mice during their forced swim session

Chronic exposure to forced swimming produced a significant increase in immobility period in control mice, the maximum response attained on day 21. Fatigue caused the increase in immobility period (*p<0.05)* in FS group as compared to normal group mice. Daily administration of candesartan (1 and 2 mg/kg; *i.p.*) for 21 days reversed the mean immobility period as assessed on alternate days i.e., 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21st day of the study respectively.

Also dexamethasone (0.5 mg/kg; *i.p.*) pre-treatment for 21 days showed reduction in (*p*<0.05) IP as compared to FS group. When mice were treated with combination of candesartan and dexamethasone for 21 days before exposing them to forced swimming, there was not significant change in IP value as compared to the FS control group (**Table 1**).

Table 1. Effect of candesartan on immobility period in different groups of FS

Groups	Treatment	Immobility time (sec)			
		1st day	7th day	13th day	21st day
FS	Saline	21 ± 1.1	131 ± 1.7[a]	229 ± 0.8[a]	323 ± 1[a]
ARB1	1 mg/kg; *i.p.*	17 ± 0.3	119 ± 1.2[b]	200 ± 1.5[b,f]	316 ± 0.7[b]
ARB2	2 mg/kg; *i.p.*	24 ± 1.3	89 ± 1[c,f]	186 ± 1.1[c,f]	224 ± 0.2[c,f]
Dexa	0.5 mg/kg; *i.p.*	18 ± 0.8	81 ± 0.5[d,f]	179 ± 1.4[d,f]	231 ± 0.4[d,f]
ARB2+Dexa	2 mg/kg; *i.p.* + 0.5 mg/kg; *i.p.*	15 ± 0.2	125 ± 0.7[e,g]	215 ± 1.2[e,g]	311 ± 1[e,g]

Values are expressed as mean ± S.E.M. [a] denotes *p*<0.05 compared to day 1 in FS group, [b] denotes *p*<0.05 compared to day 1 in ARB1 group, [c] denotes *p*<0.05 compared to day 1 in ARB2 group, [d] denotes *p*<0.05 compared to day 1 in Dexa group, [e] denotes *p<0.05* compared to day 1 in ARB2+Dexa group, [f] denotes *p<0.05* compared to respective day in FS group, [g] denotes *p<0.05* compared to respective day in ARB2 group(one way ANOVA followed by Tukey's test).

The administration of LPS followed by exposure of mice to forced swimming for 21 days also produced a significant increase in immobility period in control mice, the maximum response attained on day 21.

However, the administration of ARB2 for 21 days before FS exposure in LPS + ARB2 group protected the animals from fatigue as indicated by low IP value as compared to LPS control group. When LPS group mice were treated with the combination of candesartan and dexamethasone for 21 days, there was not significant change in IP value as compared to the LPS control group (**Table 2**).

Table 2. Effect of candesartan on immobility period in different groups of LPS

Groups	Treatment	Immobility time (sec)			
		1st day	7th day	13th day	21st day
LPS	Saline	36 ± 1.1	173 ± 1.7[a]	255 ± 0.8[a]	341 ± 1.0[a]
ARB2+LPS	2 mg/kg; *i.p.* + 1 mg/kg; *i.p.*	24 ± 1.3	143 ± 1[b,d]	210 ± 1.1[b,d]	285 ±0.2[b,d]
LPS+ARB2+Dexa	1 mg/kg; *i.p.* + 2 mg/kg; *i.p.* + 0.5 mg/kg; *i.p.*	30 ± 0.8	151 ± 0.5[c,d]	239 ± 1.4[c,d]	311 ± 0.4[c,d]

Values are expressed as mean ± S.E.M. [a] denotes *p*<0.05 compared to day 1 in LPS group, [b] denotes *p*<0.05 compared to day 1 in ARB2+LPS group, [c] denotes *p*<0.05 compared to day 1 in LPS+ARB2+Dexa group, [d] denotes *p<0.05* compared to respective day in LPS group (one way ANOVA followed by Tukey's test).

Effect of candesartan on Transfer Latency (TL) in elevated plus maze

The exposure of mice to forced swim (FS) daily for 10 min for 21 days caused the increase in TL (*p<0.05)*in FS group as compared to normal group mice. However, pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) for 21 days prevented the enhancement of TL significantly (*p<0.05)* as compared to FS group. Dexamethasone (0.5 mg/kg; *i.p.*) pre-treatment

for 21 days also reduced (p<0.05) TL as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also caused the increase in TL as compared to normal group mice. However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group protected the animals from memory impairment as indicated by low TL value as compared to LPS control group. When FS as well as LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was not significant change in TL value as compared to their respective control groups i.e. FS and LPS groups; although, TL values were significantly (p<0.05) higher than FS+ARB2 and LPS+ARB2 groups. In candesartan (2mg/kg; *i.p.*) per se group, there was no significant change in TL value (8±1.71) compared to normal group (**Figure 1**).

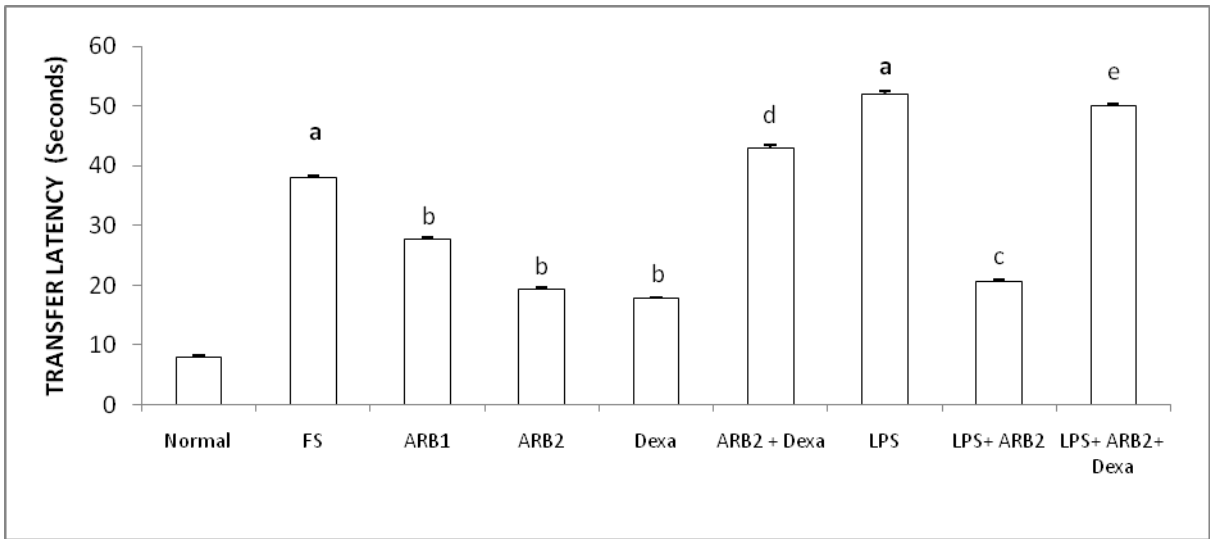

Fig. 1. Effect of Candesartan on transfer latency in elevated plus maze in mice

Values are expressed as mean ± S.E.M. [a] denotes p<0.05 compared to normal group, [b] denotes p<0.05 compared to FS group, [c] denotes p<0.05 compared to LPS group, [d] denotes p<0.05 compared to ARB2 group, [e] denotes $p<0.05$ compared to LPS+ARB2 group.

Effect of candesartan on elevated zero maze parameters

Mice were forced to swim (FS) daily for 10 min for 21 days resulted in induction of CFS. These mice when exposed to EZM showed increased LEO value ($p<0.05$) as compared to normal group mice. Pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) and dexamethasone (0.5 mg/kg; *i.p.*) separately for 21 days exerted the anxiolytic activity in mice which is shown by reduction in LEO significantly ($p<0.05$) as compared to FS group. Further, administration of LPS followed by exposure of mice to forced swimming for 21 days also lead to increase in LEO as compared to normal group mice which is significantly attenuated by the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group. When FS as well as LPS group mice were treated with combination of candesartan and dexamethasone for 21 days, there was no significant change in LEO value as compared to FS and LPS groups respectively; although, LEO values were significantly (p<0.05) higher than FS+ARB2 and LPS+ARB2 groups. There was significant decrease in LEO (151±0.9) in candesartan per se group compared to normal group.

FS group mice showed decrease in NEO ($p<0.05$) as compared to normal group mice. However, pre-administration of separate groups of mice with candesartan (1 and 2 mg/kg; *i.p.*) and dexamethasone (0.5 mg/kg; *i.p.*) for 21 days prevented the decrease in NEO significantly ($p<0.05$) as compared to FS group.

The administration of LPS followed by exposure of mice to forced swimming for 21 days also decreased the NEO as compared to normal group mice. However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group prevented the decrease in NEO as compared to LPS control group.

When FS as well as LPS group mice were treated with combination of candesartan and dexamethasone for 21 days, there was no significant change in NEO as compared to their respective control groups; although, NEO was

significantly (p<0.05) lower than FS+ARB2 and LPS+ARB2 groups. There was significant increase in NEO (18±0.6) in candesartan per se group compared to normal group.

FS induced CFS in mice caused the decrease in TEO (*p<0.05)* as compared to normal group mice. However, pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) for 21 days increased TEO significantly (*p<0.05)* as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also decreased TEO as compared to normal group mice.

However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group improved anxiety by increasing TEO as compared to LPS control group. Dexamethasone (0.5 mg/kg; *i.p.*) pre-treatment for 21 days also increased (p<0.05) TEO as compared to FS group.

When FS as well as LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was no significant change in TEO value as compared to their respective control groups; although, TEO values were significantly (p<0.05) lower than FS+ARB2 and LPS+ARB2 groups. There was significant increase in TEO (110±1.3) in candesartan per se group compared to normal group (**Table 3**).

Table 3. Effect of candesartan on elevated zero maze parameters

Groups	LEO (Sec)	NEO	TEO (Sec)
Normal	213±0.023	14±0.3073	86±0.023
FS	242±0.156[a]	8±0.3073[a]	58±0.156[a]
ARB1	217±0.345[b]	11±0.5[b]	83±0.345[b]
ARB2	187±0.122[b]	17±0.444[b]	102±0.122[b]
Dexa	203±0.154[b]	18±0.432[b]	97±0.154[b]
ARB2+Dexa	245±0.372[d]	12±0.3333[d]	55±0.372[d]
LPS	266±0.524[a]	4±0.4014[a]	34±0.524[a]
ARB2+LPS	212±0.222[c]	13±0.4[c]	95±0.222[c]
LPS+ARB2+Dexa	268.3±0.121[e]	9±0.4284[e]	31.7±0.121[e]

Values are expressed as mean ± S.E.M. [a] denotes p<0.05 compared to normal group, [b] denotes p<0.05compared to FS group, [c] denotes p<0.05 compared to LPS group, [d] denotes p<0.05 compared to ARB2 group, [e] denotes *p<0.05* compared to LPS+ARB2 group.

Effect of candesartan on ambulation score of mice in open field

There was significant reduction in locomotor activity (p<0.05) when the mice were forced to swim (FS) daily for 10 min for 21 days in FS group as compared to normal group mice. Pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) and dexamethasone (0.5 mg/kg; *i.p.*) separately for 21 days prevented decrease in ambulation significantly (*p<0.05)* as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also decreased the ambulatory score as compared to normal group mice which is significantly increased by the administration of ARB2 daily for 21 days before forced swimming in LPS+ARB2 group.

When FS as well as LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was no significant change in ambulation score and locomotor activity falls below normal as compared to their respective control groups. There was no significant change in ambulation score (153±0.84) in candesartan per se group compared to normal group (**Figure 2**).

Effect of candesartan on tail-withdrawal latency (TWL) in tail-immersion test

Sensitivity towards pain increased in stressed mice as compared to normal mice. When mice were exposed to forced swimming (FS) daily for 10 min for 21 days there was decrease in TWL (*p<0.05)* in FS group as compared to normal group mice.

However, pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) for 21 days prevented the decrease in TWL significantly (*p<0.05)* as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also caused the decrease in TWL as compared to normal group mice.

However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group protected the animals from hyperalgesia as indicated by high TWL value as compared to LPS control group. Dexamethasone (0.5 mg/kg; *i.p.*)

pre-treatment for 21 days also increased (p<0.05) TWL as compared to FS group.
When FS as well as LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was no significant change in TWL value as compared to their respective control groups; although, TWL values were significantly (p<0.05) lower than FS+ARB2 and LPS+ARB2 groups. The TWL in candesartan per se group was not significant (8±1.1) compared to normal group (**Figure 3**).

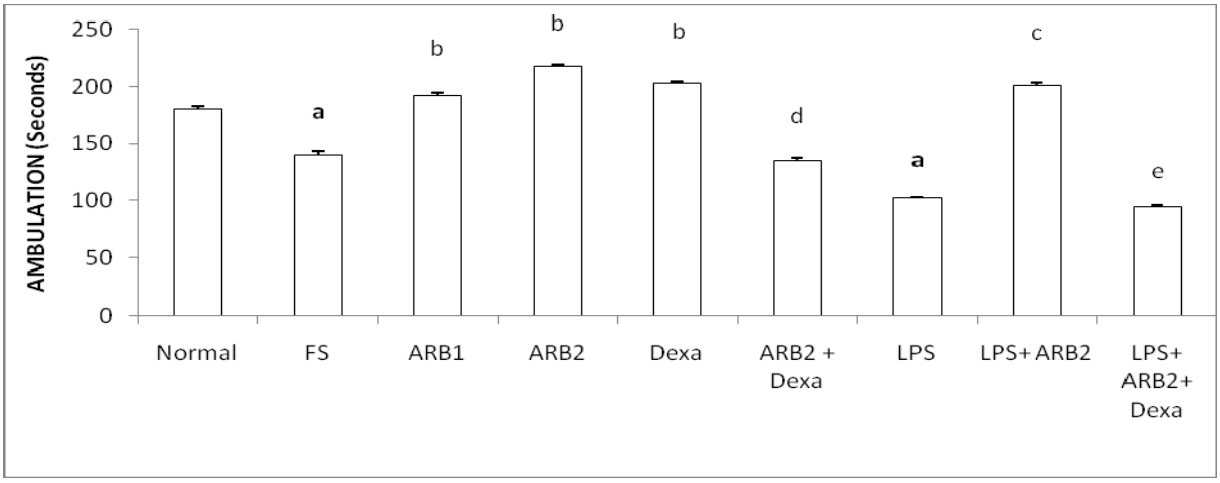

Fig. 2. Effect of candesartan on ambulation in stress exposed mice

Values are expressed as mean ± S.E.M. [a] denotes p<0.05 compared to normal group and [b] denotes p<0.05compared to FS group, [c] denotes p<0.05 compared to LPS group, [d] denotes p<0.05 compared to ARB2 group, [e] denotes *p<0.05* compared to LPS+ARB2 group.

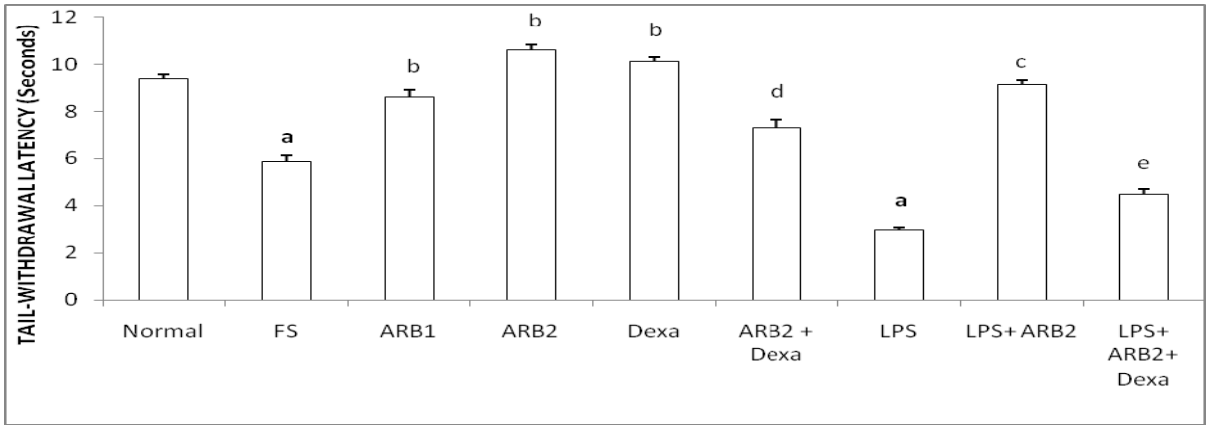

Fig. 3. Effect of candesartan on tail-withdrawal latency time in tail-immersion test

Values are expressed as mean ± S.E.M. [a] denotes p<0.05 compared to normal group and [b] denotes p<0.05compared to FS group, [c] denotes p<0.05 compared to LPS group, [d] denotes *p<0.05* compared to ARB2 group, [e] denotes *p<0.05* compared to LPS+ARB2 group.

Effect of candesartan on brain TBARS levels in mice

Chronic exposure to forced swimming (FS) daily for 10 min for 21 days produced a significant (*p<0.05*) increase in brain TBARS level in FS group as compared to normal group mice. Daily administration of candesartan (1 and 2 mg/kg; *i.p.*) and dexamethasone (0.5 mg/kg; *i.p.*) separately for 21 days prevented the elevation in brain TBARS level significantly (*p<0.05)* as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also caused the elevation in brain TBARS level as compared to normal group mice. However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group protected the animals from oxidative stress as indicated by low brain TBARS value as compared to LPS control group. When FS as well as LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was not

significant change in brain TBARS value as compared to their respective control groups *i.e.* FS and LPS groups; although, brain TBARS values were significantly (p<0.05) higher than FS+ARB2 and LPS+ARB2 groups. There was significant reduction in brain TBARS (0.93±0.77) in candesartan per se group compared to normal group (**Table 4**).

Effect of candesartan on brain GSH levels in mice

Mice chronically subjected to forced swimming (FS) daily for 10 min for 21 days showed a significant (p<0.05) decrease in the whole brain reduced glutathione levels as compared to normal group mice.

However, pre-treatment of mice with candesartan (1 and 2 mg/kg; *i.p.*) for 21 days prevented the attenuation of brain GSH level significantly (*p<0.05)* as compared to FS group. The administration of LPS followed by exposure of mice to forced swimming for 21 days also caused the attenuation of brain GSH level as compared to normal group mice. However, the administration of ARB2 for 21 days before FS exposure in LPS+ARB2 group protected the attenuation of reduced glutathione as indicated by elevated brain GSH value as compared to LPS control group.

Dexamethasone (0.5 mg/kg; *i.p.*) pre-treatment for 21 days also enhanced (p<0.05) brain GSH levels as compared to FS group. When FS and LPS administered group mice were treated with combination of candesartan and dexamethasone for 21 days, there was not significant change in brain GSH value as compared to their respective control groups; although, brain GSH values were significantly (p<0.05) lower than FS+ARB2 and LPS+ARB2 groups. There was no significant change in the brain GSH (3.57±1.2) in candesartan per se group compared to normal group (**Table 4**).

Table 4. Effect of candesartan on brain TBARS and GSH levels

Groups	Treatment	TBARS (μM/ml)	GSH (μM/ml)
Normal	Saline	1.299 ±0.053	3.3±0.012
FS	Saline	1.745 ±0.123[a]	2.3±0.021[a]
ARB1	1 mg/kg; *i.p.*	1.44 ±0.041[b]	2.782±0.0171[b]
ARB2	2 mg/kg; *i.p.*	1.225 ±0.023[b]	3.123±0.024[b]
Dexa	0.5 mg/kg; *i.p.*	1.212±0.111[b]	2.941±0.0233[b]
ARB2+Dexa	2 mg/kg; i.p. + 0.5 mg/kg; *i.p.*	1.54±0.056[d]	1.93±0.015[d]
LPS	Saline	2.225±0.121[a]	1.53±0.014[a]
ARB2+LPS	2 mg/kg; *i.p.* + 1 mg/kg; *i.p.*	1.7±0.042[c]	2.659±0.013[c]
LPS+ARB2+Dexa	1 mg/kg; *i.p.* + 2 mg/kg; *i.p.* + 0.5 mg/kg; *i.p.*	2.01±0.021[e]	1.32±0.014[e]

Values are expressed as mean ± S.E.M. [a] denotes p<0.05 compared to normal group and [b] denotes p<0.05compared to FS group, [c] denotes p<0.05 compared to LPS group, [e] denotes p<0.05 compared to FS group, [e] denotes *p<0.05* compared to LPS+ARB2 group (one way ANOVA followed by Tukey's test).

DISCUSSION

During an immune response, an important reciprocal relationship between immune products and brain function occurs. This cross talk between the immune system and brain is essential to maintain homeostasis (Elenkov *et al* 2000). Chronic stress has been associated to cause anxiety-like behaviour, reduced locomotor activity (Kumar *et al* 2010) impaired memory and stress induced hyperalgesia (Dhir and Kulkarni, 2008). The present study aimed at investigating the probable role of Angiotensin receptor blocker (Candesartan) in the management of chronic fatigue syndrome in rodents. It has been demonstrated that renin-angiotensin modulating drugs play beneficial role in attenuating stress-associated anxiety. The development of anxiety has been associated with activation of AT_1 receptors and therefore major attention has been paid to selective AT_1 receptor antagonists (Bali and Jaggi, 2013). Although AT_1 receptor blockers are well known for their cardiovascular activity therefore, they may prove to be beneficial in stress associated fatigue by maintaining blood pressure of a fatigued patient. Forced swimming and bacterial endotoxin (lipopolysaccharide) are useful tools for inducing chronic fatigue syndrome in rodents (Gupta *et al* 2009). In the present study, chronic fatigue in mice was induced by exposing mice to forced swimming daily for 10 min for successive 21 days and also by the administration of single

dose of LPS followed by equivalent volume of saline. It was found that immobility period of CFS mice increased to maximum on day 21. The level of fatigue was evaluated by various behavioural (EPZ, EZM, Open field test, tail-immersion test) and biochemical estimations (blood glucose and cortisol levels, brain TBARS and GSH levels) that confirmed the occurrence of chronic fatigue in mice. Further, administration of LPS also produced robust increase in the immobility duration, which reached peak levels on the 21st day. This model elucidates long-term behavioural and pathophysiological consequences of immune activation (Gupta *et al* 2009). Our results of FS and LPS groups confirmed the successful induction of CFS and these CFS mice showed reduced ambulatory activity in locomotor test (open field), increased anxiety in EZM, impaired memory in EPM, and increased sensitivity to pain in tail-immersion test, increased brain TBARS levels and reduced brain GSH levels. The results of elevated zero maze presented here describe a possible anxiolytic effect of candesartan as shown by the increased number of entries in open arm and enhancement in total time spent in open arm. Likewise, in elevated plus maze, transfer latency has been taken as an index of memory impairment. Decrease in transfer latency showed recovery from a memory dysfunction and candesartan treated mice exhibited decrease in transfer latency. In open field test, candesartan treated mice showed an increased ambulation in open field indicating increased exploratory behaviour; however CFS mice showed decreased ambulation. In tail-immersion test, there was hyperalgesic response in chronically fatigued mice which was attenuated by daily treatment with candesartan. We found a significant correlation between the aforementioned behavioural parameters and the levels of oxidative stress markers such as brain TBARS and GSH. Our data demonstrated a significant effect of candesartan pre-treatment as cerebro-protective agent in oxidative stress by reducing TBARS levels while increasing GSH levels and improving the cellular antioxidant status in mice brain, which strongly supports the hypothesis that candesartan administration plays role in management of oxidative stress in chronic fatigue syndrome.

In the present study, we report a possible anti-oxidant effect of candesartan administration in brain of mice, as demonstrated by decreased TBARS levels and increased levels of reduced GSH against chronic fatigue due to forced swimming and stress induced by LPS. Candesartan prevented the various behavioural and biochemical alterations due to chronic forced swimming and LPS induced fatigue, thus, providing the evidence regarding its beneficial effects in chronic fatigue syndrome. It is well reported that oxidative stress induced by LPS administration stimulated the HPA axis resulting in increased production of corticosterone (Henri *et al* 2005). When the body experiences a stressor (physical or mental), cortisol is released. Cortisol release is controlled by the hypothalamus, when it releases corticotropin releasing hormone (CRH). The CRH then affects the pituitary gland and it releases ACTH. This ACTH then causes the adrenal glands to react to the stress and release cortisol from the adrenal cortex. The amount of cortisol released by the body is controlled by a feedback loop that results from the interaction between the levels of cortisol and the hypothalamus and pituitary gland. In other words if the hypothalamus or pituitary gland sense that there is too much cortisol in the body, then they will not release as much CRH or ACTH. In Chronic Fatigue patients this negative feedback loop may be overly sensitive. When the body is stressed and needs to produce more cortisol, the hypothalamus and pituitary gland are too sensitive to the cortisol and do not produce enough CRH or ACTH. Pretreatment of mice with dexamethasone (a synthetic drug of the corticosteroid) and candesartan simultaneously caused reversal of protective effect of candesartan, which confirms involvement of HPA axis dysfunction in the pathogenesis of chronic fatigue syndrome. Candesartan selectively blocks Angiotensin II AT_1 receptors in brain thus prevents angiotensin-II from stimulating adrenal cortex. ARBs directly decrease the pro-inflammatory effects of IL-1β in neurons, including reduction of IL-1β receptor upregulation, NADPH oxidase activation, ROS production, JNK and c-Jun activation, and pro-inflammatory COX-2/PGE2 (Anderson, 2010; Erhardt *et al* 2006). ARBs may not only reduce production of excessive pro-inflammatory factors, but also decrease neuronal vulnerability to injury (Khoury *et al* 2012). These properties are of significant clinical value, and help to explain the increasing evidence that treatment with ARBs ameliorates the incidence and progression of acute and chronic neurodegenerative conditions such as the Alzheimer's disease and stroke, in which neuro-

inflammation plays an important role (Sanchez-Lemus *et al* 2012).

CONCLUSION

From present study, it can be concluded that candesartan exerts broad beneficial effects and thus may be a potentially valuable therapeutic agent for treating stress related disorders. However, more elaborated studies are needed to explore the detailed route of action.

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