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# Experimental Induction of Anxiety in Albino Mice and Its Modulation by Some Antianxiety Agents

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## ABSTRACT

**Background:** *The anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components. These components combine to create an unpleasant feeling that is typically associated with uneasiness, apprehension, fear, or worry.*

**Objectives:** *The present study was designed to investigate possible anxiolytic effects of angiotensin II (AT1) receptor antagonist, losartan, and angiotensin–converting enzyme (ACE) inhibitor, captopril using the elevated plus-maze (EPM), and the open field, a behavioural tests for anxiolytic drugs. Furthermore, the effects of these drugs will be compared with diazepam to determine whether the behavioural profile of losartan and captopril differs from this established anxiolytic drug.*

**Material and Methods:** *An experimental study in albino mice was undertaken to probe probable role renin-angiotensin system (RAS) in the genesis of anxiety. Elevated plus-maze and open-field models were used. The standard antianxiety drug-diazepam and drugs modifying RAS-captopril and losartan were used to modify the anxiety like behaviour. Cessation of chronic diazepam or ethanol caused withdrawal-induced anxiety.*

**Results:** *In comparison, cessation of captopril or losartan did not show any withdrawal effects. Acute or repeated administration of α2-antagonist-yohimbine (as anxiogenic drug) produced anxiety like behaviour Losartan and captopril individually antagonized the anxiety like behaviour due to the withdrawal of diazepam. Losartan alone antagonized the anxiety like behaviour due to the withdrawal of ethanol while captopril did not. Diazepam, losartan, and captopril individually antagonized the anxiety-like behaviour due to the administration of yohimbine in. Chronic co-treatment with diazepam and the above RAS modifying drugs afford complete suppression of diazepam withdrawal-induced effect (increased fecal boli, and decreased central ambulation) in the open field and decreased time spent on the open arms and decreased the number of entries on to the open arms in EPM.*

**Conclusion:** *It can be concluded that decreasing activity of RAS either by decreasing the synthesis of angiotensin II (captopril) or blocking of AT1 receptors (losartan), antagonizes the anxiety induced-like behaviour. Losartan was better than captopril in this regard. The possible mechanism may include RAS interaction with NA or GABA neurons, hormonal release, potentiation of AT2 receptors, and possible involvement of bradykinin levels due to captopril. Further studies to probe into the possible involvement of the above mechanisms are envisaged.*

***Keywords:*** *Anxiety, Animal anxiety test, Elevated plus maze, Open field apparatus, Diazepam, Losartan, Captopril, Ethanol, Yohimbine.*

## INTRODUCTION

There are adjustment disorders with anxiety features, and disorders due to general medical conditions and substance-induced anxiety disorders [5]. The anxiety is associated with three or more of the following symptoms for at least 6 months: restlessness, feeling keyed up or on edge, being easily fatigued, difficulty in concentrating or mind going blank, irritability, muscle tension, sleep disturbance, and irritability [1].

Elevated plus-maze is the simplest apparatus to study the anxiolytic response of almost all types of antianxiety agents. Exposure of the animals to novel maze alley evokes an approach avoidance conflict, which is stronger in the open arm as compared to the enclosed arm. Rodents (rats and mice) have an aversion for high and open space and prefer enclosed arm and, therefore, spend a greater amount of time in enclosed arm. When animals enter an open arm, they freeze, become immobile, defecate and show fear-like movements. The plasma cortisol

level is also reported to be increased, as a true reflection of anxiety [4, 6]. The open field test (OFT) is a common measure of exploratory behavior both qualitatively and quantitatively. The most basic and common outcome of interest is "movement"; however, this can be influenced by motor output, exploratory drive, freezing or other fear-related behavior, sickness, relative time in the circadian cycle, among many other variables [4, 7, 8].

## Objectives

Angiotensin II on intracerebroventricular (icv) administration produced an anxiogenic effect in animal models. There is an increasing interest in the potential role of the angiotensin II in normal brain function and in CNS disorders, with particular focus on anxiety and depression. The present study was designed to investigate possible anxiolytic effects of angiotensin II ($AT_1$) receptor antagonist, losartan, and angiotensin–converting enzyme (ACE) inhibitor, captopril using the elevated plus-maze (EPM), and the open field, behavioural tests for anxiolytic drugs. Furthermore, the effects of these drugs will be compared with diazepam to determine whether the behavioural profile of losartan and captopril differs from this established anxiolytic drug.

## Material and Methods

### Materials

#### *Animals*

Male albino mice weighing (27±3 g), were obtained from the animal house of Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Tripoli University, Libya. The animals were housed in colony cages, at constant room temperature (24±2°C), on a 12/12h light-dark cycle. Food and water were given ad libitum, food was obtained from ALCO, Sfax Tunisia.

#### *Drugs and Chemicals*

Losartan potassium, obtained from Merk, USA and captopril malate, diazepam, yohimbine hydrochloride, and ethanol were obtained from Sigma Aldrich, Germany. Tween 80 was obtained from Riedel-De Haen AG Seelzf-Hannover.

#### *Drugs Administration*

All drugs were dissolved in normal saline containing tween 80 (1%) (vehicle). Drugs were administered intraperitoneally (i.p.) route, at volume of 5ml/kg body weight. All drugs were freshly prepared.

#### *Equipments*

#### Elevated Plus-Maze (EPM)

The apparatus was made of plexiglass and consisted of two opposite open arms (5 x 30 cm) crossed with two opposite enclosed arms of the dimension with 15 cm height. The arms were connected with a central square (5x 5 cm) to give the apparatus a plus sign appearance. The maze was kept elevated 30 cm above the floor in a dimly lit room [9-12].

#### Open field

This test utilizes behavioural changes in rodents exposed to a novel environment and has been used to detect an anxiogenic or anxiolytic activity under identical situations. Various types of open-field apparatuses have been used for rats and mice. A typical apparatus suitable for mice, comprises of area, (50 cm x 50 cm x 40.5 cm). The floor is divided into 16 squares by clear lines and the apparatus is placed in a dimly-lit room [10, 13].

### Methods

#### *Elevated Plus-Maze Test*

The elevated plus-maze test was conducted in a closed room with a low level of illumination [12], the mice were individually placed on the central square of the plus maze facing an enclosed arm. The time spent and number of entries made by the mice, during the next 4 min on open and closed arm was recorded. An arm entry was defined when all the four limbs were on the arm. The apparatus was cleaned after each use. An increase in the open arm entries and an increase in time spent in the open arms is indicative of potential anxiolytic activity, as mice naturally prefer the closed arms. This technique is rapid, selective, and equally capable of detecting anxiolytic and an anxiogenic drug effects under identical conditions [9-11].

#### Effect of Diazepam, Losartan, and Captopril on the Anxiogenic Response of Ethanol Withdrawal Using the Elevated Plus-Maze

The effect of losartan, captopril, and standard anxiolytic drug diazepam on the anxiogenic response of ethanol withdrawal was examined using the elevated plus-maze test in mice. Mice were randomly assigned to four groups (n=8, each group). All groups of mice received ethanol (8% w/v, 5 ml/kg) (i.p) twice daily (9-10am and 6-7pm), for fourteen days then withdrawn for 24 hrs. Anxiogenic response

induced by ethanol withdrawal was investigated by losartan, captopril, and standard anxiolytic drug diazepam during the period of withdrawal. Group2, diazepam (1.5 mg/kg) [14-17], and group3, losartan (10 mg/kg) [13, 17], were administered at 12 hrs and then 30 min before testing 24 hrs after withdrawal. Similarly, group4, captopril (10 mg/kg) [16, 18] was given at 12 hrs and then at 45 min before the test. All drugs were freshly prepared, and administered in a constant volume of injection 5 ml/kg body weight. All above three groups were compared with group 1, which received vehicle 12 hrs and 30 min before the test. All groups were exposed to elevated plus-maze test on day fifteen.

### Effect of Diazepam, Losartan, and Captopril on an Anxiogenic Effects of Acute Dose of Yohimbine Using the Elevated Plus-Maze

This study was performed to investigate the effect of an acute dose of losartan, captopril, and standard anxiolytic drug diazepam on an anxiogenic action of acute dose of yohimbine (2.5 mg/kg IP) [15, 19, 20], in mice using the elevated plus-maze test. Mice were divided into four groups (n=8, each group), group1 (yohimbine + vehicle); group2 (yohimbine + diazepam); group3 (yohimbine + losartan), and group4 (yohimbine + captopril). Drugs were administered intraperitoneally (i.p), in a constant volume of injection of 5 ml/kg body weight. Anxiogenic response induced by yohimbine was investigated by losartan, captopril, and anxiolytic drug diazepam. Losartan (10 mg/kg), anxiolytic drug diazepam (1.5 mg/kg), and yohimbine (2.5 mg/kg) were injected 30 min before the test, captopril (10 mg/kg) was injected 45 min before the test. All treated groups were compared with group1, which received yohimbine + vehicle. Elevated plus-maze test was performed as previously described.

### Effect of Diazepam, Losartan, and Captopril on Anxiogenic Effects of Repeated Treatment with Yohimbine Using the Elevated Plus-Maze

This study was performed to investigate the effect of an acute dose of losartan, captopril, and standard anxiolytic drug diazepam on an anxiogenic action of repeated treatment with yohimbine (2.5 mg/kg IP for 5 days). Elevated plus-maze test was used as model to conduct this experiment. Mice were randomly assigned to four groups (n=8). All groups of mice received yohimbine (2.5 mg/kg i.p) twice daily (9-10am and 6-7pm), for five days. Anxiogenic response induced by repeated administration of yohimbine was investigated by losartan, captopril and standard anxiolytic drug diazepam. On the test day (day five), yohimbine was administered 30 min before testing in all groups. At day five group1 received vehicle; group2 and group3 received diazepam (1.5 mg/kg ip) or losartan (10 mg/kg ip) 30 min before the test respectively, whereas group4 received captopril (10 mg/kg) 45 min before the test. Then all animals were exposed to elevated plus-maze test.

### Effects of Captopril and Losartan on the Diazepam Dependence and their Dependence-Liability Using the Elevated Plus-Maze

The experiment was performed to investigate the effects of captopril and losartan on the diazepam dependence in mice. In addition, captopril and losartan have also been evaluated for their dependence-liability in mice upon a chronic treatment–regimen (14-days). Elevated plus-maze test was used as model to conduct this experiment. The animals were divided into vehicle and different treated groups (n=8). The treated groups received diazepam (10 mg/kg ip), losartan (10 mg/kg ip), captopril (10 mg/kg ip), diazepam (10 mg/kg ip) + losartan (10 mg/kg ip), and diazepam (10 mg/kg ip) + captopril (10 mg/kg ip), respectively twice daily (at 9-10am and 6-7pm) for 14 days. No drug was administrated for the next 24 hours. At day fifteen, all animals were tested with elevated plus-maze test.

#### *Open Field Test*

In the open field, each animal was centrally placed in the test apparatus for 4 min, and the following behavioural aspects of anxiety were recorded:

1. Central ambulation - This was measured in terms of the number of squares crossed by the animals in the four central squares of the open field.

2. Defecation - The number of fecal boli excreted during the period.

Since exposure to a novel environment is associated with emotionality, an anxious animal is one which shows reduced ambulation associated with periodic freeze, concomitant with augmented autonomic activity resulting in increased defecation. All these effects are accentuated by anxiogenic agents and attenuated by anxiolytics. The open-field test is simple, sensitive, and reproducible, and appears to be similarly influenced by different classes of anxiogenic and anxiolytic agents [10, 13].

### Effect of an Acute Dose of Diazepam, Captopril and Losartan on Anxiety Parameters Using Open Field

The effect of captopril (10 mg/kg), losartan (10 mg/kg), and diazepam (1.5 mg/kg), on anxiety parameters evaluated in albino mice using open field test as described before. Mice were randomly assigned to four groups (n=8, each group). Losartan ,captopril and diazepam were administered i.p. to mice. Control group received an equivalent volume of vehicle i.p. Fourty five min after administration of captopril and 30 min after administration of losartan, diazepam and vehicle, four groups were tested on the open field test.

### Effects of Captopril and Losartan on the Diazepam Dependence and their Dependence-Liability Using Open Field

In the present experiment, we examined the effect of captopril and losartan on the diazepam dependence in mice. In addition, captopril and losartan have also been evaluated for their dependence-liability in mice upon a chronic treatment–regimen (14-days). Open field test was used as model to conduct this experiment. The animals were divided into vehicle and different treated groups (n=8). The treated groups received diazepam (10 mg/kg ip), losartan (10 mg/kg ip), captopril (10 mg/kg ip), diazepam (10 mg/kg ip) + losartan (10 mg/kg ip), and diazepam (10 mg/kg ip) + captopril (10 mg/kg ip), respectively twice daily (at 9-10am and 18-19pm) for 14 days. No drug was administrated for the next 24 hours. At day fifteen, all animals were tested with open field test.

### *Statistical Analysis*

Descriptive statistical analysis was performed, on the parameters of samples within each experiment, to find out whether the observed samples were normally distributed, using the non parameteric Kolmogorove-smirnov maximum deviation test for goodness of fit. If the parameters were normally distributed, the treatments were compared by applying one-way ANOVA, according to the homogeneity of variance, data were transferred in trank if homogeneity of variance did not permit direct ANOVA analysis. For multiple compression Post hoc, additional the least significant difference (LSD) tests were performed, when appropriate, to detect any significant differences between the treated groups and the control group, and between the combined drugs and drug itself. The difference were considered to be significant at ($p<0.05$). All analyses were conducted using the SPSS (software packing version 25) for IBM compatible computer.

## RESULTS

### Modulation of Ethanol Withdrawal-Induced Anxiogenic Action by Administration of Diazepam and Losartan Using the Elevated Plus-Maze

#### *Effects on Time Spent on Open Arms*

The mean duration of time spent in the open arms of vehicle "control" treated group (30.125 ± 6.057 sec) which was significantly different from losartan (84.125 ± 18.828 sec) and diazepam (75.75 ± 13.027 sec) treated groups. In contrast, the time spent by captopril treated group (39.25 ± 5.28 sec) was insignificantly different from vehicle treated group. (Table. 1).

**Table1.** *Effects of diazepam, losartan and captopril on the ethanol withdrawal-induced anxiogenic action using the elevated plus maze*

| Treatments (mg/kg) | Time spent in the open arms (second) | Number of entries in the open arms |
|---|---|---|
| Vehicle (control) | 30.125 ± 6.057 | 3.125 ± 0.398 |
| Diazepam (1.5) | 75.75 ± 13.027* | 6.5 ± 0.597* |
| Losartan (10) | 84.251 ± 18.828* | 7 ± 1.558** |
| Captopril (10) | 39.250 ± 5.283 | 3.75 ± 0.559 |

*N = 8 animals in each group. Statistical significance: *p<0.05, **p<0.01. All data are mean values with (±S.E.M) and compared with control.*

#### Effects on Number of Entries on Open Arms

The number of entries in the open arms of vehicle "control" treated group (3.125 ± 0.398) which was highly significantly different from losartan (7±1.55), and significantly different from diazepam (6.5 ± 0.597) treated groups respectively. In contrast, the number of entries by captopril treated group (3.75 ± 0.55) was insignificantly different from control group (Table.1).

### Acute Administration of Yohimbine-Induced Anxiety and its Modulation by Diazepam, Losartan and Captopril Using the Elevated Plus-Maze

#### *Effects on Time Spent on Open Arms*

The mean duration of time spent in the open arms of yohimbine + vehicle treated "control" group (30.125 ± 4.954 sec) which was highly significantly different from yohimbine + diazepam (90.375 ± 18.73 sec) but significantly different from yohimbine + captopril (77.75 ± 21.10 sec) and yohimbine + losartan (77.5 ± 8.9 sec) treated groups respectively (Table .2).

*Effects on Number of Entries on Open Arms*

The number of entries to open arms of group treated with yohimbine + vehicle "control" group (2.25 ± 0.163) which was significantly different from yohimbine + diazepam (6.375 ± 1.831), yohimbine + captopril (5.5 ± 0.981) and yohimbine + losartan (6.25 ± 0.7007) treated groups (Table.2).

**Table2.** *Effects of diazepam, losartan and captopril on the acute dose of yohimbine-induced an anxiogenic action using the elevated plus maze.*

| Treatments (mg / kg) | Time Spent in the open arms (seconds) | Number of entries in the open arms |
|---|---|---|
| Yohimbine (2.5) + vehicle | 30.125 ± 4.9549 | 2.250 ± 0.1636 |
| Yohimbine (2.5) + diazepam (1.5) | 90.375 ± 18.733 ** | 6.375 ± 1.831 * |
| Yohimbine (2.5) + losartan (10) | 77.500 ± 8.9022 * | 6.250 ± 0.7007 * |
| Yohimbine (2.5) + captopril (10) | 77.7500 ± 21.104 * | 5.5 ± 0.9891 * |

*N = 8 animals in each group. Statistical significance: *p<0.05, **p<0.01, respectively. All data are mean values with (±S.E.M) and compared with yohimbine + vehicle treated "control" group.*

## Repeated Administration of Yohimbine-Induced Anxiety and its Modulation by Diazepam, Losartan and Captopril Using the Elevated Plus- Maze

*Effects on Time Spent on Open Arms*

The mean duration of time spent in the open arms of yohimbine + vehicle treated "control" group (24.625 ± 5.070 sec) which was significantly different from yohimbine + captopril (58.750 ± 9.1666 sec), yohimbine + losartan (60.750 ± 12.637sec) and yohimbine + diazepam (66.625 ± 15.337 sec) treated groups (Table .3).

*Effects on Number of Entries on Open Arms*

The number of entries to open arms of group treated with yohimbine + vehicle "control" group (1.625 ± 0.263) which was significantly different from yohimbine + diazepam (4.5 ± 1.165), yohimbine + losartan (4.125 ± 0.4407) and yohimbine + captopril (4.125 ± 0.6665) treated groups (Table.3).

**Table3.** *Effects of diazepam, losartan and captopril on the repeated administration of yohimbine-induced an anxiogenic action using the elevated plus-maze.*

| Treatments (mg / kg) | Time spent in the open arms (seconds) | Number of entries in the open arms |
|---|---|---|
| Yohimbine (2.5) + vehicle | 24.625 ± 5.07 | 1.625 ± 0.263 |
| Yohimbine (2.5) + diazepam (1.5) | 66.625 ± 15.337 * | 4.5 ± 1.165* |
| Yohimbine (2.5) + losartan (10) | 60.750 ± 12.637* | 4.125 ± 0.4407* |
| Yohimbine (2.5) + captopril (10) | 58.75 ± 9.166* | 4.125 ± 0.666 * |

*Statistical significance: *p<0.05. All data are mean values with (±S.E.M) and compared with yohimbine + vehicle treated "control" group.*

## Modulation of Diazepam Dependence-Induced Severe Anxiety by Losartan and Captopril and their Dependence-Liability Using the Elevated Plus-Maze

*Chronic Treatment with Diazepam Induced Severe Anxiety*

Chronic administration produce dependence and withdrawal effects appeared when the drug was discontinued. On abrupt termination of diazepam after 14-days treatment, the animals show relapse of severe anxiety. The maximum withdrawal effects (sever anxiety) were monitored using the elevated plus-maze test 24 hours after the cessation of 14-days treatment with diazepam (i.e on the 15$^{th}$ day).

Effects on Time Spent on Open Arms

The mean duration of time spent in the open arms of diazepam treated group (30.125 ± 5.920 sec) which was highly significantly different from vehicle treated group (65.5 ± 9.615 sec) (Table.4).

Effects on Number of Entries on Open Arms

The number of open arm entries of diazepam treated group (3.125 ± 0.350) which was highly significantly different from vehicle treated group (6.375 ± 0.625) (Table.4).

*Chronic Treatments with Losartan and Captopril Did Not Induce Anxiety*

Chronic administration of losartan (10 mg/kg ip b.i.d.) and captopril (10 mg/kg ip b.i.d.) for 14 days did not produce any anxiety like behaviour. After the discontinuation of 14-days treatment with losartan and captopril, were monitored

using the elevated plus-maze test 24 hours after the cessation of 14 -days, treatment.

### Effects on Time Spent on Open Arms

The mean duration of time spent in the open arms of vehicle treated group (65.5 ± 9.615 sec) which was insignificantly different from losartan (59.5 ± 5.756 sec) and captopril (54.25 ± 6.878 sec) treated groups.

In contrast, the mean duration of time spent in the open arms of diazepam treated group (30.125 ± 5.920 sec) which was highly significantly different from losartan (59.5 ± 5.756 sec) and significantly different from captopril (54.25 ± 6.878 sec) treated groups (Table.4).

### Effects on Number of Entries on Open Arms

The number of open arms entries of vehicle treated group (6.375 ± 0.625) which was insignificantly different from losartan (6 ± 0.534) and captopril (5.5 ± 0.5976) treated groups.

In contrast, The number of open arm entries of diazepam treated group (3.125 ± 0.350) which was highly significantly different from losartan (6 ± 0.534) and captopril (5.5 ± 0.5976) treated groups (Table.4).

### *Diazepam Dependence Induced Severe Anxiety and its Modulation by Chronic Administration of Losartan and Captopril*

Losartan (10 mg/kg ip b.i.d.) and captopril (10 mg/kg ip b.i.d.) upon concurrent administration with 10 mg/kg diazepam for 14 days, significantly prevented the anxiety like behaviour due to the withdrawal of diazepam, as monitored using the elevated plus-maze test 24 hours after the end of 14 $^{th}$ –days, treatment.

### Effects on Time Spent on Open Arms

The mean duration of time spent in the open arms of vehicle treated group (65.5 ± 9.615 sec) which was insignificantly different from captopril + diazepam (49.625 ± 5.40), and losartan + diazepam (51.625 ± 2.329) treated groups.

In contrast, the mean duration of time spent in the open arms of diazepam treated group (30.125 ± 5.920 sec) which was significantly different from losartan + diazepam (51.625 ± 2.329) and captopril + diazepam (49.625 ± 5.404) treated groups (Table.4).

### Effects on Number of Entries on Open Arms

The number of open arms entries of vehicle treated group (6.375 ± 0.625) which was insignificantly different from losartan + diazepam (5 ± 0.5345) and captopril + diazepam (4.875 ± 0.8331) treated groups.

In contrast, the number of open arms entries of diazepam treated group (3.125 ± 0.350) which was significantly different from losartan + diazepam (5 ± 0.534) and captopril + diazepam (4.875 ± 0.833) treated groups (Table .4).

**Table4.** *Effects of losartan and captopril on the diazepam dependence and their dependence-liability using the elevated plus-maze*

| Treatments (mg / kg) | Time spent in the open arms (s) | Number of entries in the open arms (n) |
|---|---|---|
| Vehicle | 65.5 ± 9.615 ** | 6.375 ± 0.625 ** |
| Diazepam (10) | 30.125 ± 5.920 | 3.125 ± 0.350 |
| Losartan (10) | 59.5 ± 5.756** | 6 ± 0.534 ** |
| Captopril (10) | 54.250 ± 6.878 * | 5.5 ± 0.597** |
| Diazepam (10) + losartan (10) | 51.625 ±2.329* | 5 ± 0.534* |
| Diazepam (10) + captopril (10) | 49.625 ± 5.404* | 4.87 ± 0.833* |

*N = 8 animals in each group. Statistical significance: *p<0.05, **p<0.01, respectively. All data are mean values with (±S.E.M) and compared with diazepam treated group.*

## Effect of an Acute Dose of Diazepam, Captopril and Losartan on Anxiety Parameters Using Open Field

### Central Ambulation

The number of central ambulation of vehicle "control" treated group (5.625 ± 0.73) which was significantly different from losartan (13.5 ± 3.02), captopril (11.75 ± 2.09) and diazepam (12.75 ± 1.64) treated groups (Table.5).

### Defecation

The number of faecal boli of vehicle "control" treated group (2.625 ± 0.323) which was highly significantly different from losartan (1.125 ± 0.35), captopril (1.25 ± 0.31) and diazepam (1 ± 0.37) treated groups (Table.5).

**Table5.** *Effects of diazepam, losartan and on anxiety parameters using open field*

| **Treatments (mg/kg)** | **Central ambulation** | **fecal boli** |
|---|---|---|
| Vehicle | 5.625 ±0.730 | 2.625 ± 0.323 |
| Diazepam(1.5) | 12.75 ± 1.644* | 1 ± 0.3779** |
| Losartan (10) | 13.5 ± 3.029* | 1.125 ± 0.350** |
| Captopril (10) | 11.75 ± 2.093* | 1.25 ± 0.313 ** |

*N = 8 animals in each group. Statistical significance: *p<0.05, **p<0.01, respectively. All data are mean values with (±S.E.M) and compared with control.*

## Modulation of Diazepam Dependence-Induced Severe Anxiety by Losartan and Captopril and Their Dependence-Liability in an Open Field

### *Effect of chronic treatment with diazepam*

Chronic administration produce dependence and withdrawal effects appeared when the drug was discontinued. On abrupt termination of diazepam after 14-days treatment, the animals show relapse of severe anxiety. The maximum withdrawal effects (sever anxiety) were monitored using the open field test 24 hours after the cessation of 14-days treatment with diazepam (i.e on the 15$^{th}$ day).

#### Central Ambulation

The number of central ambulation with diazepam treated group (1.25 ± 0.25) which was highly significantly different from vehicle treated group (5.125 ± 0.54) (Table.6).

#### Defecation

The number of faecal boli of diazepam treated group (5.25 ± 0.79) which was highly significantly different from vehicle treated group (1.85 ± 0.45) (Table.6).

### *Chronic Treatment with Losartan and Captopril Did Not Induce Anxiety*

Chronic administration of losartan (10 mg/kg ip b.i.d.) and captopril (10 mg/kg ip b.i.d.) for 14 days did not produce any dependence. After the discontinuation of 14-days treatment with losartan and captopril the animals were monitored using the open field test 24 hours after the cessation of the chronic treatment.

#### Central Ambulation

The number of central ambulation of vehicle treated group (5.125 ± 0.54) which was insignificantly different from losartan (4.5 ± 0.62) and captopril (4.37 ± 0.679) treated groups.

In contrast, mice treated with diazepam showed (1.25 ± 0.25) of central ambulation which was significantly different from losartan (4.5 ± 0.62) and captopril (4.37 ± 0.679) treated groups (Table.6).

#### Defecation

The number of faecal boli of vehicle treated group (1.85 ± 0.45) was insignificantly different from losartan (2.14 ± 0.40) and captopril (2.71 ± 0.521) treated groups.

In contrast, the number of faecal boli of diazepam treated group (5.25 ± 0.79) which was very highly significantly different from losartan (2.14 ± 0.40) and highly significantly different from captopril (2.71 ± 0.521) treated groups respectively (Table.6).

### *Modulation of Diazepam Dependence Induced Sever Anxiety by Chronic Administration of Losartan or Captopril*

Losartan (10 mg/kg IP b.i.d.) and captopril (10 mg/kg IP b.i.d.) upon concurrent administration with 10 mg/kg diazepam for 14 days, significantly prevented the anxiety like behaviour due to the withdrawal of diazepam, as monitored using the open field test 24 hours after the end of 14$^{th}$ –days, treatment.

#### Central Ambulation

The number of central ambulation of vehicle treated group (5.125 ± 0.54) was insignificantly different from losartan + diazepam (4.125 ± 0.718) and captopril + diazepam (3.87 ± 1.02) treated groups.

In contrast, mice treated with diazepam showed (1.25± 0.25) of central ambulation which was significantly different from losartan + diazepam (4.125 ± 0.718) and captopril + diazepam (3.87 ± 1.02) treated groups (Table.6).

#### Defecation

The number of faecal boli of vehicle treated group (1.85 ± 0.45) was insignificantly different from losartan + diazepam (2.75 ± 0.453) and captopril + diazepam (3 ± 0.597) treated groups.

In contrast, the number of faecal boli of diazepam treated group (5.25 ± 0.79) which was highly significantly different from losartan + diazepam (2.75 ± 0.453) and captopril + diazepam (3 ± 0.597) treated groups (Table.6).

**Table6.** *Effects of losartan and captopril on the diazepam dependence and their dependence- liability using open field.*

| Treatments (mg /kg) | Central ambulation | Fecal boli |
|---|---|---|
| Vehicle | 5.125 ± 0.548** | 1.857 ± 0.459*** |
| Diazepam (10) | 1.25 ± 0.25 | 5.25 ± 0.796 |
| Losartan (10) | 4.5 ± 0.626* | 2.142 ± 0.404*** |
| Captopril (10) | 4.375 ± 0.679* | 2.71 ± 0.521** |
| Diazepam (10) + losartan (10) | 4.125 ± 0.718* | 2.75 ± 0.453** |
| Diazepam (10) + captopril (10) | 3.875 ± 1.025* | 3 ± 0.597** |

*N = 8 animals in each group. Statistical significance: *p<0.05, **p<0.01, ***p<0.001, respectively. All data are mean values with (±S.E.M) and compared with diazepam treated group.*

## DISCUSSION

The role of renin angiotensin system (RAS) within CNS with functional analysis of $AT_1$ receptor and Angiotensin converting enzyme (ACE) was examined. The results revealed that the RAS plays important role in regulation of anxiety-like behaviour by inhibiting ACE or blocking angiotensin receptor.

The elevated plus-maze (EPM) and the open field tests were used as validated animal models of anxiety that are predictive of drug responses in humans. These tests, which are based on natural aversion of rodents to open spaces, are sensitive to the effects of both anxiolytic and anxiogenic agents in rodents [16]. The elevated plus-maze was developed for use in rats by Handley and Mithani, [21] following the work of Montgomery, [22], and was Subsequently modified for use in the mouse independently by Lister [9] and Stephens *et al.,* [23]. These various adaptations of the basic method have since been used to study the anxiolytic actions of many drugs such as benzodiazepines [19], a NMDA receptor antagonist [23], neuroactive steroids, captopril [18], and losartan [17].

The open field is a similar exploratory model of anxiety, which was initially described by Kulkarni and Dandiya, [24], Kulkarni *et al.,* [25] and later developed and validated by Kulkarni, [26]. This test has too been used to investigate a range of putative anxiolytic compounds such as diazepam [10] and losartan [13].

The renin angiotensin system (RAS) plays an important role in the control of the balance of hydromineral and fluid volume, as well as sympathetic efferent activity [27]. The octapeptide angiotensin II is a potent effector hormone of the RAS produced in the mammalian brain [28, 29]. It exerts a wide range of physiological actions on the cardiovascular, renal and endocrine system [30], and on the peripheral and central nervous system [31] by acting on different angiotensin II receptor types. Angiotensin exerts its effects via two receptor subtypes, namely $AT_1$ and $AT_2$ receptors [32]. It is the $AT_1$ receptors that are the most abundant and which mediate most of the physiological responses to angiotensin, with $AT_2$ receptors being concerned with longer-term effects such as cardiac myocyte proliferation. Both receptor subtypes have been identified in the brain, although $AT_1$ receptors account for approximately 90% of the population [33]. There is an increasing interest in the potential role the ocatapeptide angiotensin II in normal brain function and in CNS disorders, with particular focus on anxiety and depression. The effect of angiotensin II can be controlled either by inhibiting ACE or blocking angiotensin II receptor. Combined therapy may enable complete blockade, but it has shown toxic symptoms in normal conditions [34]. Losartan is a nonpeptide orally active antihypertensive, selective $AT_1$ receptor antagonist act by interfering with the RAS via $AT_1$ receptor blockade [35]. Angiotensin II on intracerebroventricular (icv) administration produced an anxiogenic effect in the open-field behaviour, and it was reversed with losartan treatment. Further, in normal subjects also, losartan produced anxiolytic effect [36]. The $AT_1$ receptor antagonist was found to modulate the mental function and produced anxiolysis in mice [37]. Captopril is an ACE inhibitor that inhibit the conversion of angiotensin I to active angiotensin II in body tissues and used extensively in the treatment of hypertension and heart failure [18]. ACE inhibitor, captopril, producing mood elevating effects in patients prescribed as a drug for hypertension [38-40], and has been reported to produced anxiolytic behaviour in humans [13].

In the EPM time spent on the open arms of the maze and the number of entries onto the open arms are taken as measures of anxiety [23]. Anxiolytic drugs increase both of these parameters. The established anxiolytic agent diazepam produced a significant increase in the amount of time spent on the open arms of the maze and the number of entries onto the open arms [17]. Anxiolytic property of losartan has also been reported in different behavioural paradigms [36, 41]. This suggests a possible relationship between $AT_1$ receptor function and anxiolytic response. A similar effect has been reported in treatment with captopril [18]. This suggests a possible relationship between ACE function and anxiolytic response. In the present study treatment with losartan and captopril significantly increased the the amount of time spent on the open arms of the maze and the number of entries onto the open arms which indicates the role of angiotensin in the altered behaviour.

Drug withdrawal-induced anxiety. This particular test has been used during recent years on the basis that endogenously generated

anxiety is likely to provide a better and clinically acceptable animal model of anxiety [15]. It is known that drug withdrawal, after chronic administration of addictive agents, induces severe withdrawal symptoms of anxiety in rodents [11]. The following drugs have been used ethanol (8% w/v, i.p.), cocaine (1 mg/kg, i.p.), nicotine (0.1 mg/kg, i.p.), diazepam (10 mg/kg, i.p.) and morphine (10 mg/kg, i.p.). The drugs were administrated twice daily for 14 days [42]. Anxiety is assessed 24 hrs after drug withdrawal by EPM [42]. Since this anxiety is endogenously generated, it can be regarded as more physiological and clinically relevant than anxiety induced by anxiogenic agents [11]. From previous studies ACE inhibitors could reverse the anxiety induced with RAS activation [43], anxiety induced by abrupt cessation of chronic treatment with a proven anxiolytic drugs which may be attributed to the escalation of RAS. The released angiotensin could precipitate anxiogenicity, and it can also stimulate both central and peripheral sympathetic systems, including adrenal medulla [44, 45]. Activation of sympathetic nervous system increases noradrenalin turnover in the brain nuclei which are involved in noradrenergic control of body function [46].

In normal animals or after angiotensin II on intracerebroventricular administration of angiotensin peptides denotes reversal of suppressed behaviour of mice in light-dark test with angiotensin receptor antagonist [37], reversal of sympathetic hyperactivity in rats with losartan [47], anxiolytic effect of losartan in mice [17], and of ACE inhibitors in rats [48] which support our present findings. Therefore, it can be stated that the observed anxiolytic behaviour with losartan and captopril may be attributed to the attenuation of the inherent anxiogenic behavior in normal mice and to antagonizing angiotensin-mediated neuro chemical alteration in anxiouse mice. In the present study, the effect of endogenous anxiety, generated by the withdrawal of certain drugs known to induce dependence was investigated. The present results provide evidence that captopril and losartan may have anxiolytic potential, indicating anxiety as a consequence of increased RAS ton. Hence, it can be assumed that increased sympathetic activity and direct action of angiotensin system may be responsible for the observed anxiogenicity after an abrupt cessation of a chronic treatment with diazepam.

The mouse model has also proven useful to demonstrate behavioural changes following withdrawal from treatment with ethanol and diazepam. An abrupt cessation of chronic treatment is associated with a relapse of anxiety, which is manifested in the form of decreased amount of time spent and the number of entries onto the open arms as measured by the elevated plus-maze test [11] and in the present studies, both captopril and losartan antagonised the anxiety like behaviour of withdrawal from diazepam and, losartan also antagonised the anxiety like behaviour of withdrawal from ethanol while captopril did not, indicating direct blocking of $AT_1$ receptor may have better effect in attenuation the anxiogenicity in anxious mice rather than inhibiting the enzyme action. Hence, $AT_1$ receptor blockade produces beneficial effect in controlling the altered behaviour and may be attributed to their better central effect and to presynaptic regulation of the neurotransmitters release.

Many symptoms of ethanol withdrawal are due to increased activity of the sympathetic nervous system, including hypertension, tremor and anxiety [49]. The $\alpha_2$ agonists clonidine [50] and medetomidine [51] are considered to be effective in the manangement of ethanol withdrawal symptoms. In our study losartan fully reversed the anxiogenic effects of ethanol withdrawal in the EPM, whereas captopril failed to reverse ethanol withdrawal-induced anxiety-like behaviour in the EPM, which suggest that direct blocking of $AT_1$ receptor may have better effect to reverse the anxiogenic effects of ethanol withdrawal rather than inhibiting the enzyme action. Thus, the possible explanation for the anxiolytic-like behavior we observed in the present study with losartan, while did not with captopril, may be due to attenuated sympathetic neurotransmission because of the ablocking $AT_1$ receptor with losartan, whereas captopril have non specific mechanism, Ang II can be formed by alternative non-ACE pathways; hence, ACE inhibitors may not provide total inhibition of angiotensin II generation [52]. In contrast, angiotensin II ($AT_1$) receptor antagonists will block angiotensin II regardless of its route of biosynthesis [52].

Benzodiazepines (e.g., diazepam, nitrazepam, lorazepam, alprazolam) are the most frequently prescribed synthetic chemical drugs for a variety of conditions, particularly anxiety, depression, epilepsy and insomnia [53, 54]. The prevalent prescription pattern of benzodiazepines is symptomatic which results in their potential overuse [55]. Chronic use of benzodiazepines causes deterioration of cognitive functioning [56], physical dependence [57] and tolerance [58, 59].

From previous studies in rodents, an abrupt cessation of a chronic treatment with a proven anxiolytic agent (e.g., ethanol or diazepam) is manifested in the form of increased anxiety [56, 57], that has a direct bearing on the locomotor behavior of the animals [60-62]. In several studies on behavioral manifestation of ethanol and diazepam withdrawal in rodents, it has been hypothesized that an abrupt cessation of chronic treatment with ethanol and diazepam leads to a relapse of anxiety, which is manifested in the form of decreased amount of time spent on the open arms of the maze and the number of entries onto the open arms as measured by the EPM [11]. The results of the present study of withdrawal from treatment with diazepam for 14 days can induced anxiety-like behaviour in the mouse elevated plus-maze test. The time spent and the number of entries on the open arms of the EPM after cessation of diazepam treatments, being statistically different from the vehicle treated group. In contrast, that administration of losartan or captopril for 14 days was not associated with such changes which is apparent from not significant differenent from vehicle treated group, even after the cessation of losartan or captopril treatments for the next 24 hours. Co-treatments of diazepam (10 mg/kg) concomitantly with the losartan (10 mg/kg) or captopril (10 mg/kg) for 14 days, also attenuated the development of diazepam withdrawal. The co-treatment of diazepam with losartan or captopril affords the complete suppression of the withdrawal-induced-anxiety, as the time spent and the number of entries on the open arms after this co-treatment is statistically equivalent to that the time spent and the number of entries on the open arms exhibited by vehicle treated group of animals on the 15 days. This is therefore an important observation that both captopril and losartan completely prevented the anxiety like behaviour of ceasing the treatment with diazepam in the EPM. This is consistent with results from previous studies with captopril [18].

Yohimbine is, a naturally occuuring alkaloid with antagonistic activity due to the alpha 2 adrenoreceptor [20]. It has been found to induce anxiety in man [63, 64] and in animals, it is found to reduce the amount of time spent in the open arm and the number of entries to the open arm in the elevated plus-maze [19-21].

Corticotropin-releasing factor (CRF), a 41 amino acid residue peptide, that regulates the release of ACTH from the anterior pituitary [65], has been shown to mediate stress-induced changes in the autonomic nervous system, neuroendocrine functions, and behavior [65-67]. The sngiotensin II on intracerebroventricular administration of CRF to laboratory animals produces anxiety-like behavior, such as altered locomotor activity [68] and an increased anxiety in an elevated plus-maze [69]. In our data with yohimbine, acute and repeated administration, significantly reduce the amount of time spent and the number of entries to the open arm in the elevated plus-maze. These effects indicate that yohimbine possesses anxiogenic properties; which is consistent with results from previous studies in the elevated plus-maze [19, 21]. In previous studies, acute and repeated treatment with yohimbine increased plasma corticosterone levels [19, 20]. In addition, yohimbine may have directly affected HPA function through its action on the noradrenergic system. In rodents, noradrenaline is generally thought to exert an inhibitory effect on CRF and hence, ultimately, on corticosterone secretion [70]. It is generally believed that noradrenaline exerts this effect by stimulation of the α-adrenoceptor, possibly at the $\alpha_2$ site [70], yohimbine would therefore be expected to block this inhibitory effect through antagonistic activity at this site. Therefor, the anxiety-like behavior seen in yohimbine treated mice might be related to abnormalities in the central noradrenergic neuronal system but not in the CRF neuronal system.

There is extensive evidence from previous animal work, that direct activation of the locus coeruleus which may reflect dysfunction of inhibitory $\alpha_2$ adrenoceptor which can produce signs of arousal that strongly resemble those seen in human anxiety states [71]. In case of the mouse [72, 73], the distribution of $AT_1$ receptors is similar to that observed in the rat. However, the locus coeruleus of the rat was shown to have the $AT_2$ receptor also [74]. Angiotensin II binding sites were detected in mice, and angiotensin II binding sites in the mouse locus coeruleus was sensitive to the $AT_1$-specific antagonist losartan [72]. In the present study acute and repeated administration of $\alpha_2$ adrenoceptor antagonist yohimbine induced a significant anxiogenic effect as indicated by the reduction of amount of time spent and the number of entries to the open arm in the elevated plus-maze, acute and repeated co-treatments of yohimbine concomitantly with the diazepam, losartan or captopril induced a significant increase in these variables, antagonised the anxiety and prevented the behavioural changes induced by yohimbine in the mouse model.

The amygdala appears to play a crucial role in conditioned fear and probably anxiety [75]. Both angiotensin II ($AT_1$ and/or $AT_2$) and norepinephrine receptors are located in the amygdala [72, 73, 76]. Thus, the anxiety-like behaviour observed in present results after acute and repeated administration of yohimbine may be mediated by interaction between the $\alpha_1$ adrenoceptor and angiotensin II ($AT_1$ and/or $AT_2$) receptors in the amygdala. In fact, the stress-induced anxiety-like behavior may reflect excessive norepinephrine activity via the central $\alpha_1$ adrenoceptor due to block of inhibitory effect of $\alpha_2$ adrenoceptor on norepinephrine release by yohimbine [77]. This supports the view that anxiety disorders may be, at least in part, related to excessive norepinephrine activity [71]. Moreover, functional interaction between angiotensin II and the neuronal noradrenergic system may occur [78-80].

The results in the present investigation provide no evidence of kindling or sensitization to the effects of yohimbine following 5 days of repeated pretreatment. In the elevated plus-maze the anxiogenic response was unchanged from that following acute administration.

In the present study in normal mice, the drugs acting on RAS-losartan, and captopril displayed increased ambulatory activity in center of the open field, with less fecal boli and immobility period which indicates the role of angiotensin in the altered behaviour.

The number of fecal boli in rodents is considered to be an index of their emotionality [81, 82], and the reduction in the number of fecal boli after diazepam administration, a drug widely prescribed for treatment of anxiety and related disorders [71] is seen to be consistent with its anxiolytic action [83]. Thus, an exaggerated response to a novel environment is consistent with an increase in anxiety-like state after drug withdrawal. In our study effects of captopril or losartan on the diazepam dependence in the open field, like in EPM withdrawal from treatment with diazepam for 14 days induced anxiety-like behaviour which is manifested in the form of decreased ambulatory activity in center of the open field, with more fecal boli and immobility period, being statistically different from the vehicle treated group. In contrast, that administration of losartan and captopril for 14 days was not associated with such changes which is apparent from not significantly differenent from vehicle treated group, even after the cessation of losartan or captopril treatments for the next 24 hours. Co-treatments of diazepam concomitantly with the losartan or captopril for 14 days, also attenuated the development of diazepam withdrawal. The co-treatment of diazepam with losartan or captopril affords the complete suppression of the withdrawal-induced-anxiety, as increased ambulatory activity in center of the open field, with less fecal boli and immobility period after this co-treatment is statistically equivalent to that of the above parameters exhibited by vehicle treated group of animals on the $15^{th}$ days. It was therefore an important observation that both captopril and losartan completely prevented the anxiety like behaviour due to the withdrawal of diazepam in the open field test.

It is unknown why ACE inhibitors and the $AT_1$ receptor antagonist exerted a differential behaviour in normal mice and anxious mice. The possible explanation is that angiotensin, potentiates sympathetic neurotransmission; hence, the blockade of angiotensin system with blockers may hyperpolarise the membrane potential of sympathetic neurons resulting in normalization of the activity. Further, angiotensin facilitates catecholamine release through $AT_1$ presynaptic receptor mechanism, and blockade of this with losartan may also have a beneficial effect [84]. The difference could also be accounted on their differences in neurohormonal activation, in bradykinin potentiation, and in the inhibition of the action of angiotensin II derived from the classical pathways [85]. Further, inhibition of ACE indirectly may prevent the activity of angiotensin both at $AT_1$ and $AT_2$ receptors. Reportes normally indicate that $AT_1$ receptors are involved in the mediation of anxiety [13]. $AT_2$ receptors have been reported to have opposing effects to $AT_1$ receptors [86]. Furthermore, $AT_2$ receptor Knockout mice exhibited anxiogenesis, or in other words, increased $AT_2$ receptor activity could result in anxiolytic activity [16]. Therefore, selective blockade of $AT_1$ receptor by losartan could produce better anxiolytic activity than captopril, as captopril will indirectly decrease angiotensin activity on both $AT_1$ and $AT_2$ receptors. Therefore, It can be concluded that RAS has a significant role on behaviour, and losartan has shown better effect than captopril in reversing the anxiogenicity in the normal and anxious mice, which indicates that a specific blockade of $AT_1$ receptor produces a far more pronounced effect than blocking the precursor enzyme by ACE inhibitors. The differential effect of $AT_1$

receptor blockade in anxious animal and normal animal is simply due to the fact that in normal mice the RAS is not hyperactive while it is in anxious animal.

The reason behind the anxiolytic effect may also be due to the influence of angiotensin antagonists over benzodiazepines/γ-amino butyric acid (BDZ/GABA) system. In the present study, losartan, captopril and diazepam showed a anxiolytic response in normal and anxious animals, indicating the higher tone of angiotensin and decreased BDZ/GABA system in anxiety state. From previous studies, it is evident that neurons in the hypothalamic paraventricular nucleus (PVN) are critically involved in the regulation of neuroendocrine, cardiovascular, and other physiological functions [27]. Stimulation of PVN neurons can directly and indirectly influence the sympathetic outflow and blood pressure, especially during stress and certain types of hypertension [87-89]. Both anatomical and functional evidence suggest that the PVN plays an important role in the regulation of autonomic function through Ang II [90], for instance, the sympathoexcitatory response induced by central hyperosmolality is attenuated by blockade of $AT_1$ receptors with losartan in the PVN [91]. Li *et al.,* [92] claimed that angiotensin II induced the excitation of PVN neurons, and such effect was eliminated by an $AT_1$ antagonist, losartan, but not by the $AT_2$ antagonist PD 123319. Thus, the effect of angiotensin II on PVN neurons is mediated by $AT_1$, but not through $AT_2$, receptors. Li *et al.,* [93] concluded that attenuation of GABAergic synaptic inputs contributes to Ang II-induced excitation of these PVN neurons, whereas Li et *al.,* [93] determined the effect of angiotensin II on the excitation of PVN neurons following blockade of $GABA_A$ receptors with bicuculline. Li *et al.,* [93] found that angiotensin II failed to excite the PVN neurons in the presence of bicuculline, suggesting that the excitatory effect of angiotensin II on PVN neurons is mediated by a disinhibition (reduction of GABAergic inputs) mechanism. It can be concluded that RAS is upregulated in the PVN during disease conditions such as heart failure, stress, and hypertension [94-97]. Furthermore, the blood-born angiotensin II is elevated in these pathophysiological conditions, and the circulating angiotensin II can increase the excitability of PVN neurons connecting to circumventricular organs [94, 97-99]. By attenuation of GABAergic synaptic inputs to spinally projecting PVN neurons, angiotensin II can excite these cells to augment and maintain the high level of sympathetic outflow during stress, heart failure, and hypertension [100, 101].

Biochemical and electrophysiological evidence suggests that $AT_1$ and $AT_2$ receptors have antagonistic effects. Activation of $AT_1$ and $AT_2$ receptors elicited stimulatory and inhibitory effects, respectively, on mitogen-activated protein (MAP) kinase in neurons [102]. Within the brain, blockade of periventricular $AT_2$ receptors potentiated $AT_1$ receptor-mediated stimulation of drinking and vasopressin secretion [33]. These studies collectively suggest that functional interactions between the two receptor subtypes have a key role in ANG II-induced neuromodulatory actions in neurons. An $AT_1$ receptor antagonist losartan [103] and an ACE inhibitor captopril [18], used to abolish endogenous angiotensin II, produced anxiolytic activity that suggests that the anxiolytic action may reflect inhibition of $AT_1$ receptors. Thus, one possible explanation for the anxiolytic-like behavior observed in the present study with losartan, may be the manifest activation of $AT_2$ receptor because of the blockade of $AT_1$ receptors in the CNS may cause the release of $AT_2$-receptor induced anxiolysis which may not exclude the possibility of the mutual inhibitory effect of $AT_1$ receptor. In angiotensin II-induced anxiety and blood pressure responses in $AT_2$-deficient mice, showed that $AT_2$ receptor antagonized the $AT_1$-mediated anxiogenenesis and pressor action of angiotensin II [104]. Therefore, it is possible that stimulation of exposed $AT_2$ receptors, as well as blockade of $AT_1$ receptors, may contribute to the beneficial effects of $AT_1$ receptor antagonists.

## Conclusion

It can be concluded that decreasing activity of RAS either by decreasing the synthesis of angiotensin II (captopril) or blocking of $AT_1$ receptors (losartan), antagonizes the anxiety induced-like behaviour. Losartan was better than captopril in this regard. The possible mechanism may include RAS interaction with NA or GABA neurons, hormonal release, potentiation of $AT_2$ receptors, and possible involvement of bradykinin levels due to captopril. Further studies to probe into the possible involvement of the above mechanisms are envisaged.

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