

EVALUATION OF ANXIETY AND LOCOMOTOR BEHAVIOUR IN HIGH SALT FED MICE TREATED WITH L-ARGININE AND LOSARTAN

ABSTRACT

Salt consumption above dietary guidelines is detrimental to health, it causes impaired cognitive, cardiovascular and neurodegenerative functions. This study aimed at evaluating anxiety and locomotor behaviour in high salt fed mice treated with L-arginine and losartan. Forty(40) mice weighing between 27-37g categorized into four (4) parts of ten(10) members. Group I (Control) was served ordinary mice chow and water, group II was given 8% NaCl in chow and 1% NaCl in drinkable water(High Salt Diet), group III was given same diet as group II in addition to L-arginine on the 43rd day, group IV was given same as group II in addition to Losartan on the 43rd day. L-arginine and losartan administration lasted for 14 days. Total duration for drug administration and feeding lasted for one month and three weeks. Light-Dark Transition Box (LDTB) and Elevated+ Maze (EPM) were used to assess anxiety and locomotor behaviour. The results showed a significant ($p < 0.001$) decrease in line crossing, transition frequency, light box duration, with a corresponding significant ($p < 0.001$) increase in stretch attend posture, grooming frequency duration, close arm entry, dark box duration frequency in the high salt fed group compared with the control. Both L-arginine and Losartan significantly ($p < 0.001$) reversed these changes towards normal. Thus, high salt diet is implicated in increased anxiety, whereas L-arginine, a nitric oxide precursor, and losartan, an ACE blocker reduced anxiety which in turn improves locomotor behaviour in the mice.

INTRODUCTION

L-arginine is an amino acid and constitutes building blocks of proteins. Amino acids are grouped into Non-essential amino acids that are produced in the body and Essential amino acids which is taken externally in daily meal, as such, it must be provided through dietary intake^{1,2} opined that L-arginine is sometimes referred as semi-essential because it becomes essential under certain conditions like pregnancy, infancy, critical illness, and trauma. However, it is a precursor for nitric oxide (signaling molecule) required for carrying out different body function and processes, and includes regulation of blood flow, mitochondrial, and cellular communication³. L-arginine has numerous in the body function and deficiency can disrupt cellular function as well as some organs leading to serious health challenge. L-arginine is usually synthesized from the breakdown of body proteins or produced from dietary proteins. Certain protein-rich foods like meat, poultry, dairy, nuts, soy products, and fish are concentrated with L-arginine. The daily intake of L-arginine from foods is reported to average 4–6 grams⁴.

L-arginine has versatile metabolic roles to produce several active biological intermediates substances such as nitric oxide (NO), polyamines, creatine². However, some research implicates L-arginine as a mediator of central nervous system and some of its activities are not yet fully understood. Therefore, is possible L-arginine might also affect behaviors such as anxiety and locomotor activities. Nitric oxide has been recognized to play an important role in many behavioral, cognitive and emotional processes⁵.

Anxiety and Fear are used mutually, fear refers to alertness in the presence of danger whereas, Anxiety is the unpleasant emotions evoked when fear is stimulated⁶. Anxiety could present as a serious mental ailment where patients are unable to function normally worried by every little occurrence (such as cell phone ringing or door slamming). An estimated population of 20-30% in Nigeria is assumed to suffer from mental disorders. A journal of Nutritional Neuroscience (2005) revealed that a combination of L-lysine and L-arginine ameliorate effects of stress in subjects with high trait of anxiety.

The impact of excessive dietary additives like sodium chloride is implicated in negative blood pressure and decrease in nitric oxide. High amount of sodium chloride may reduce L-arginine or decrease its cellular uptake⁷ and subsequently cause deficiency of nitric oxide Synthase cofactors⁸ and alteration in signaling pathway⁹. Inhibition or uncoupling of nitric oxide synthase will result in reduced nitric oxide production¹⁰. In hypertension, any combination of these may occur and these mal-function of the nitric oxide pathway serve to increase oxidative stress and perpetual loss of available nitric oxide.

This research therefore aimed at evaluating anxiety and locomotor behaviour in high salt fed mice treated with L-arginine and losartan.

MATERIALS AND METHODS

Some materials used include; light and dark transition box, elevated plus maze(EPM), measuring cylinder, weighing balance, stop watch, , L-arginine & losartan, Sodium chloride etc.

Experimental animals and protocol

A total of forty (40) Mice weighing 27-37g were used in the study. They were allowed access to twelve hours light and feed ad libitum. The Mice were divided into four groups with 10 rats per group. Group I as the control, was given distilled water and normal rat chow. Group II was given 8% NaCL in chow, and 1% NaCL in drinkable water. Group III was fed same as group II, but L-arginine was administered on the 43rd day. Group IV was fed same as group II, but Losartan was administered on the 43rd day of the experiment. L-arginine was administered for nine days and Losartan for four days and was done sequentially. Total duration for feeding and drug administration lasted for 56 days.

At the end of drug administration, the Light-dark transition box and Elevated plus maze were used to assessed anxiety and locomotor behaviour.

RESULTS

Frequency of Head dips in the Elevated Plus Maze among the groups.

The mean values for head dips in the Control, salt fed, salt + L-arginine and salt + Losartan group were 10.00 ± 0.17 , 6.40 ± 0.75 , 11.00 ± 0.84 and 9.20 ± 0.86 .

The results show significant decrease ($P < 0.001$) in head dips in the salt fed group. Administration of L-arginine and Losartan resulted in an increased head dips and tend towards the normal (control), Fig. 1.

Frequency of Stretch Attend Posture (SAP) among the groups.

The mean values for frequency of SAP in the control, salt fed, salt +L-arginine and salt + losartan group were 4.20 ± 0.58 , 18.80 ± 1.36 , 5.60 ± 0.71 and 8.60 ± 0.93 . The results show significant increase ($p > 0.001$) in SAP in salt fed group. Administration of L-arginine and losartan improved SAP and tends towards the normal, Fig. 2.

Grooming duration (GD) among the groups.

The GD in the control, salt fed, salt + L-arginine and salt + Losartan group were 7.60 ± 1.50 , 24.80 ± 0.24 , 3.20 ± 0.58 and 3.40 ± 0.51 respectively. The result showed a longer duration in GD of salt fed group when compared with the control. Administration of L-arginine and Losartan helped to reduce GD that tend towards the normal control, Fig. 3.

Frequency of close arm entry (CAE) among the group.

The mean values of CAE in the control, salt fed, salt + L-arginine and salt + Losartan group were 5.60 ± 1.29 , 6.40 ± 0.89 , 5.00 ± 1.14 and 4.00 ± 0.45 respectively. There was no significant difference among the groups. Perhaps, salt + Losartan group reduced CAE which indicate some specific role of Losartan, Fig. 4.

Close arm duration (CAD) among the groups.

The mean values of CAD in the control, salt fed, salt + L-arginine and salt + Losartan group were 201.60 ± 4.91 , 232.80 ± 9.47 , 201.00 ± 5.97 and 199.40 ± 6.57 respectively. The result showed a significant ($p < 0.001$) increase in CAD in the salt fed group compared with the control. Administration of L-arginine and Losartan resulted in reduced CAD, salt + Losartan group showed further reduction which may be due to specific actions of Losartan in hypertension. Fig. 5.

Line Crossing Frequency in Light/Dark Box.

The mean values of line crossing frequency in the control, salt fed, salt + L-arginine and salt + Losartan group were 98.20 ± 3.46 , 66.80 ± 4.09 , 98.40 ± 5.10 and 99.60 ± 2.66 respectively. The result showed a significant decrease ($p < 0.001$) in the

salt fed group compared with the control. Administration of L-arginine and Losartan as observed in other groups increased line crossing frequency, Fig. 6.

Stretch attend posture (SAP) frequency in light/dark box.

The mean values of SAP frequency in the Control, salt fed, salt + L-arginine and salt + Losartan group were 3.40 ± 0.51 , 13.20 ± 1.20 , 6.80 ± 0.58 and 7.40 ± 0.51 respectively. The result showed significant increase in SAP frequency in the salt fed group and was reduced in control. Administration of L-arginine and Losartan in other groups revealed decreased in SAP frequency towards normal, Fig. 7.

Light box duration (LBD) in the light/dark box test.

The mean values of LBD in the control, salt fed, salt +L-arginine and salt + Losartan group were 200.40 ± 5.89 , 262.40 ± 12.09 , 212.80 ± 3.65 and 219.40 ± 6.02 respectively. The results showed no difference compared with dark box duration (DBD), Fig. 8.

Freezing frequency (FF) in the open field maze.

The mean values of FF in the control, salt fed, salt + L-arginine and salt + Losartan group were 0.40 ± 0.24 , 1.60 ± 0.24 , 0.20 ± 0.20 and 0.40 ± 0.24 respectively. The result showed a significant increase ($p < 0.001$) in FF in the salt fed group compared to the control. Administration of L-arginine and Losartan restored the FF to normal. Fig. 9

Freezing duration (FD) in the open field maze.

The mean values of FD in the control, salt fed, salt + L-arginine and salt + Losartan group were 0.80 ± 0.49 , 9.00 ± 0.71 , 0.40 ± 0.40 and 0.40 ± 0.24 respectively.

The result showed significant increase ($p < 0.001$) in the salt fed group compared to the control. Administration of L-arginine and Losartan decreased FD towards normal.

Fig. 10.

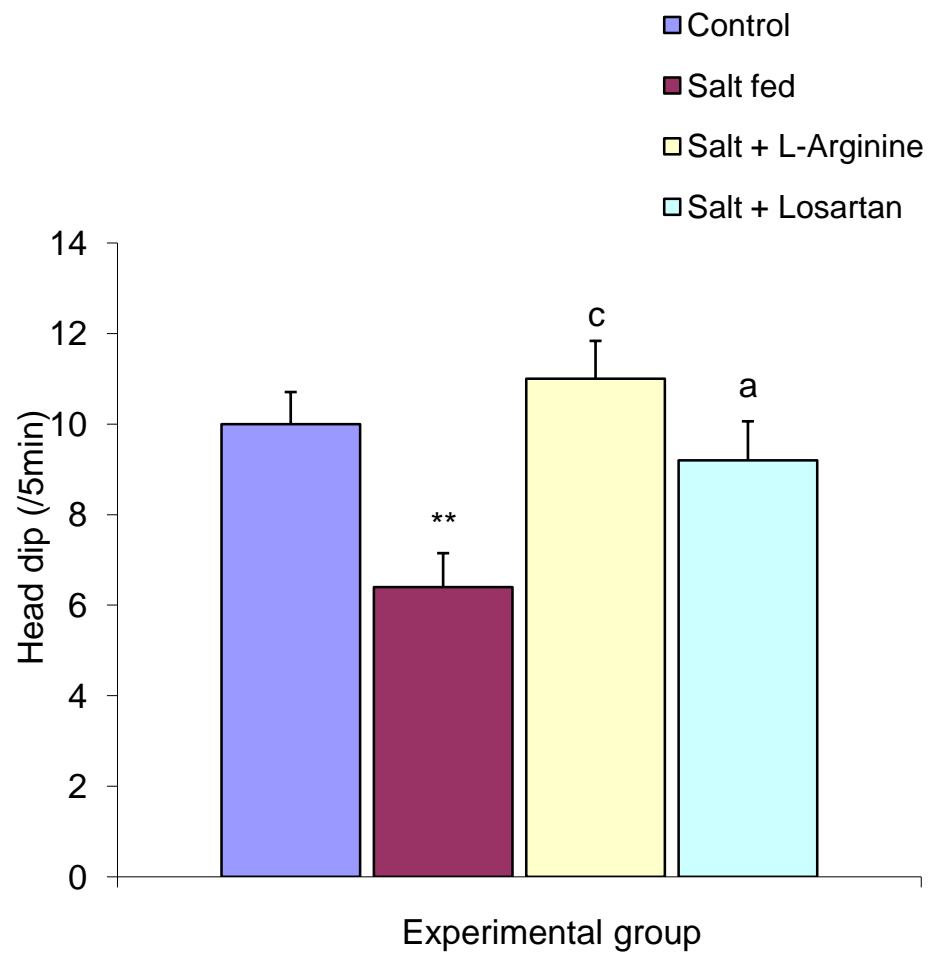


Figure 1: Frequency of head dip in the control and test groups during the elevated plus maze test.

Values are expressed as mean +SEM, n = 5.

** = $p < 0.0$ vs control

a = $p < 0.05$, c = $p < 0.001$ salt fed

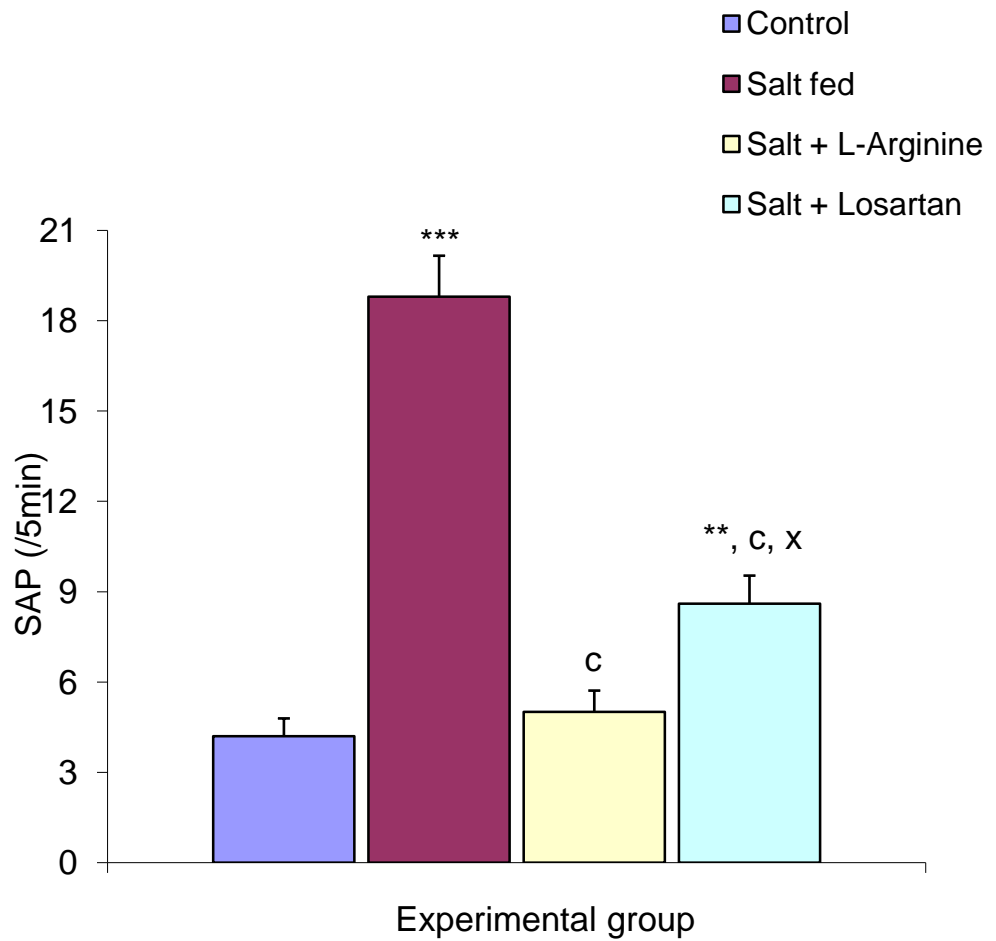


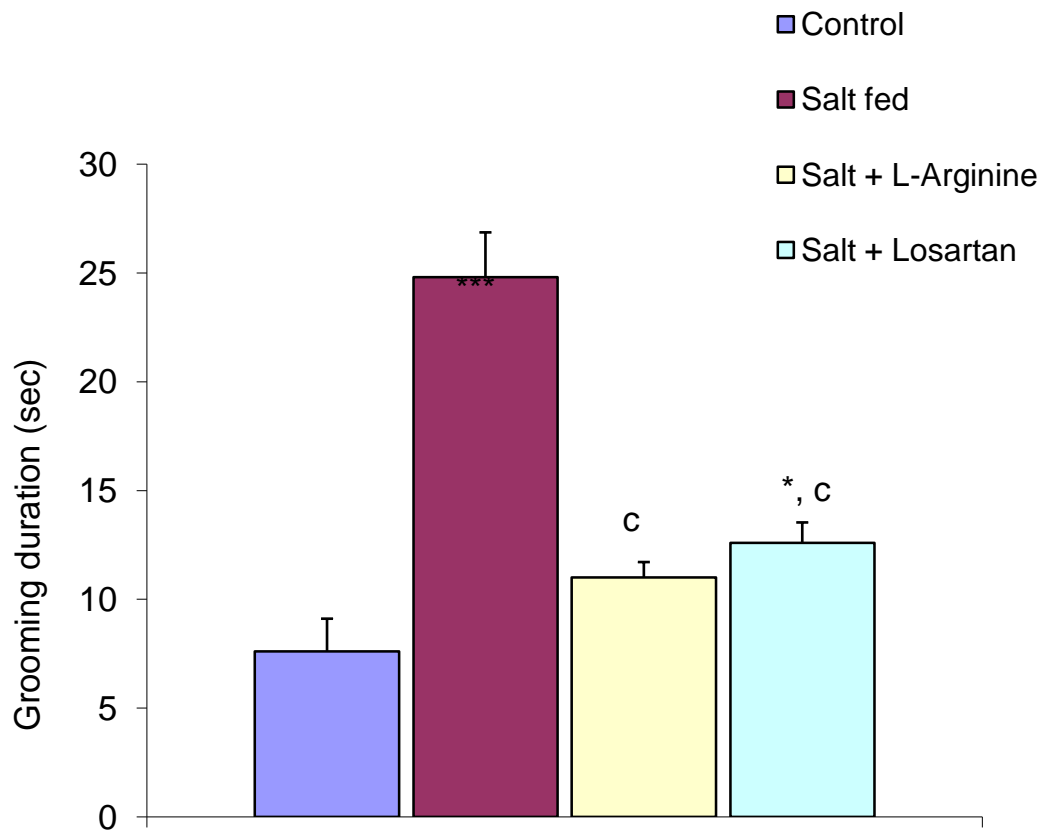
Figure 2: Stretch attend posture in the control and tests groups during the lelevated plus maze test.

Values are expressed as mean +SEM, n = 5.

** = $p < 0.01$, *** = $p < 0.001$ vs control

c = $p < 0.001$ salt fed

x = $p < 0.05$ salt + L-Arginine



Experimental group
 Figure 3: Grooming duration in the control and test groups during the elevated plus maze test.

Values are expressed as mean +SEM, n = 5.

* = $p < 0.05$, *** = $p < 0.001$ vs control

c = $p < 0.001$ salt fed

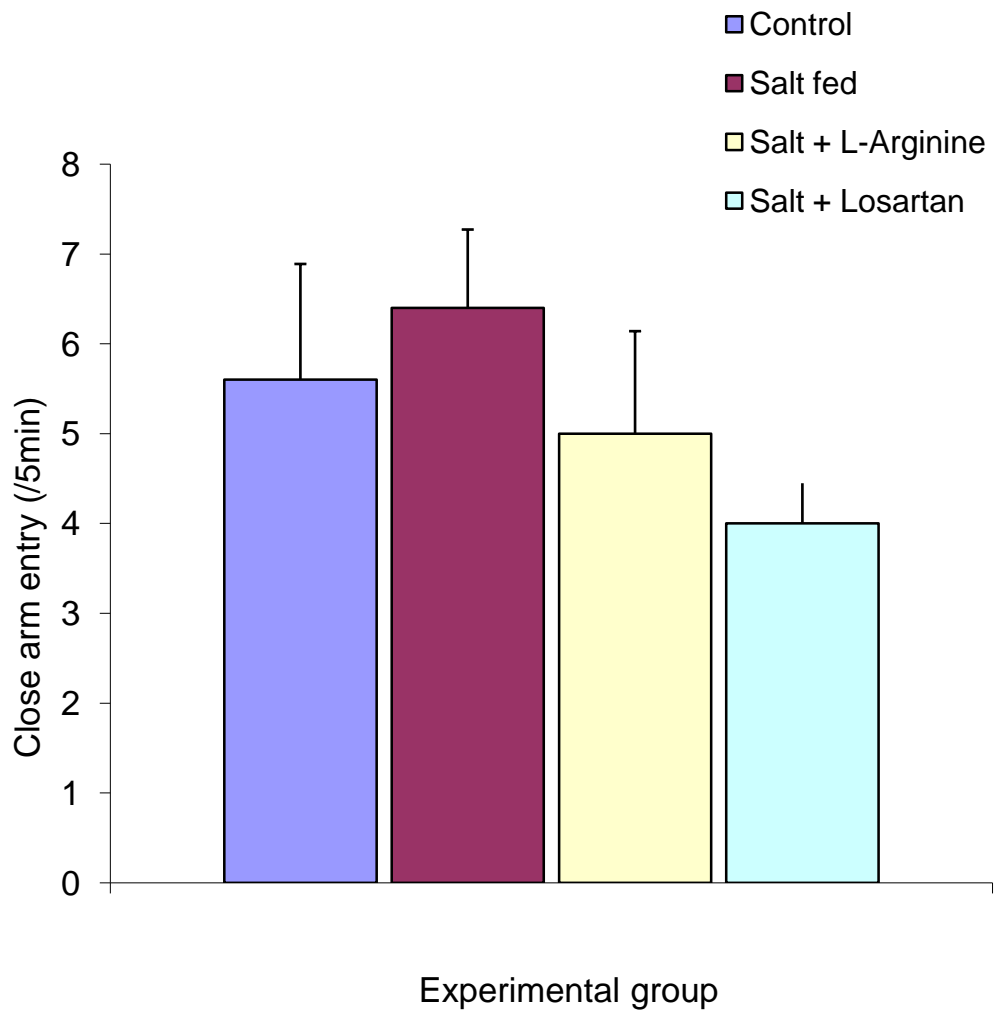
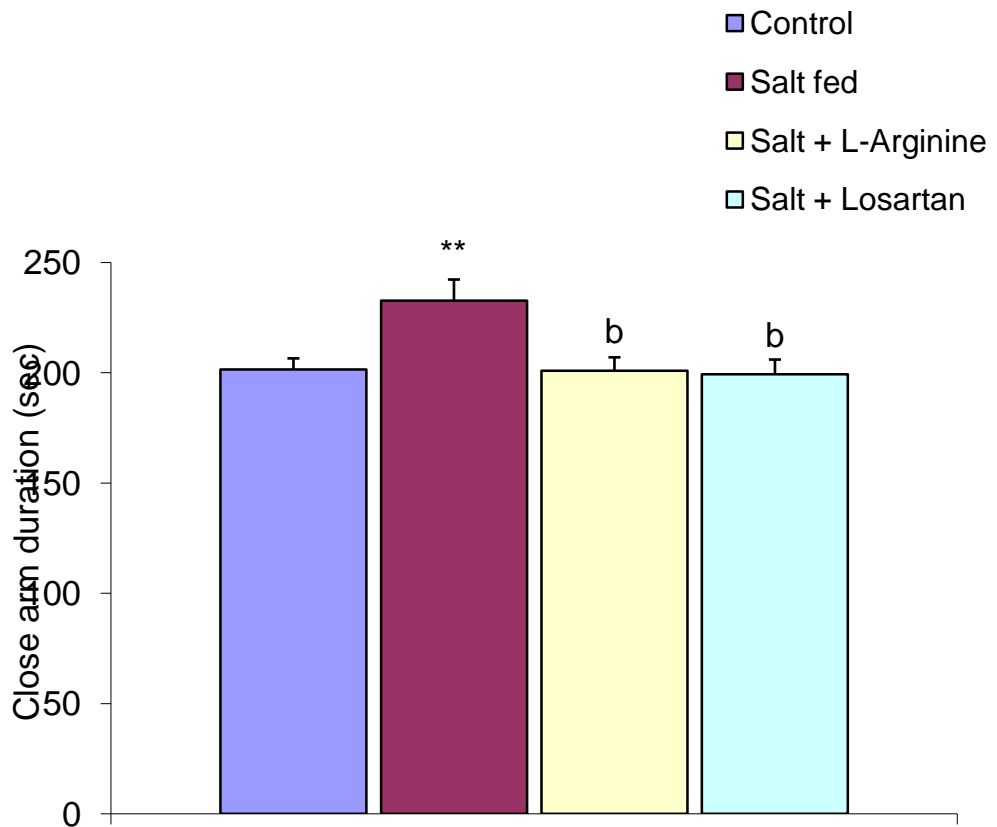


Figure 4: Frequency of close arm entry in the control and test groups during the elevated plus maze test.

Values are expressed as mean +SEM, n = 5.
No significant differences among groups



Experimental group
 Figure 5: Close arm duration in the control and test groups during the light dark transition box test.

Values are expressed as mean +SEM, n = 5.

** = p<0.01 vs control

b = p<0.01 salt fed

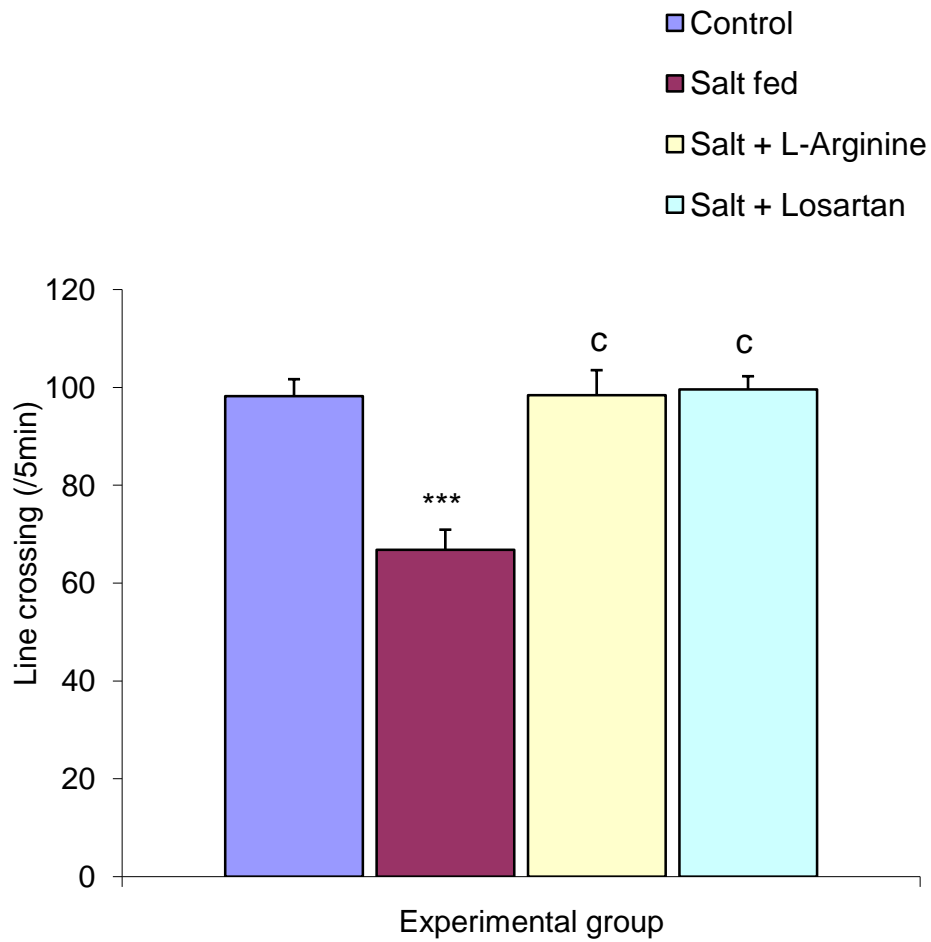


Figure 6: Line crossing in the control and test groups during the light dark transition box test.

Values are expressed as mean +SEM, n = 5.

*** = p<0.001 vs control

c = p<0.001 salt fed

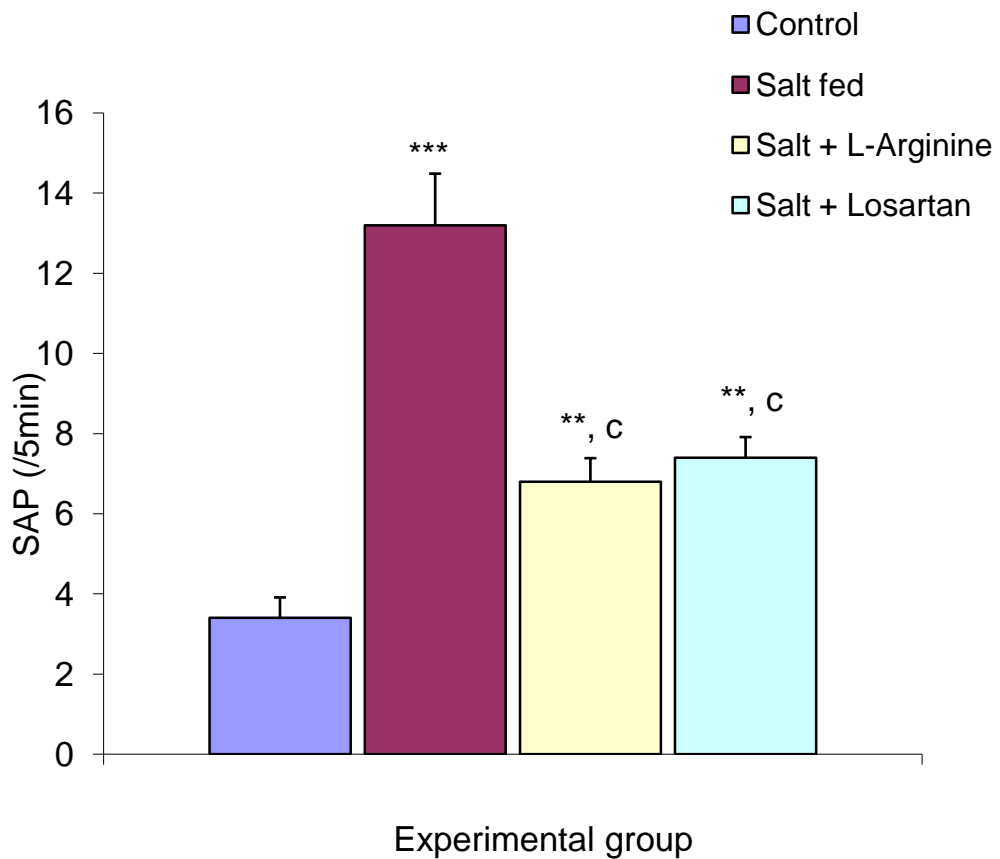


Figure 7: Stretch attend posture in the control and tests groups during the light dark transition box test.

Values are expressed as mean +SEM, n = 5.

** = $p < 0.01$, *** = $p < 0.001$ vs control

c = $p < 0.001$ salt fed

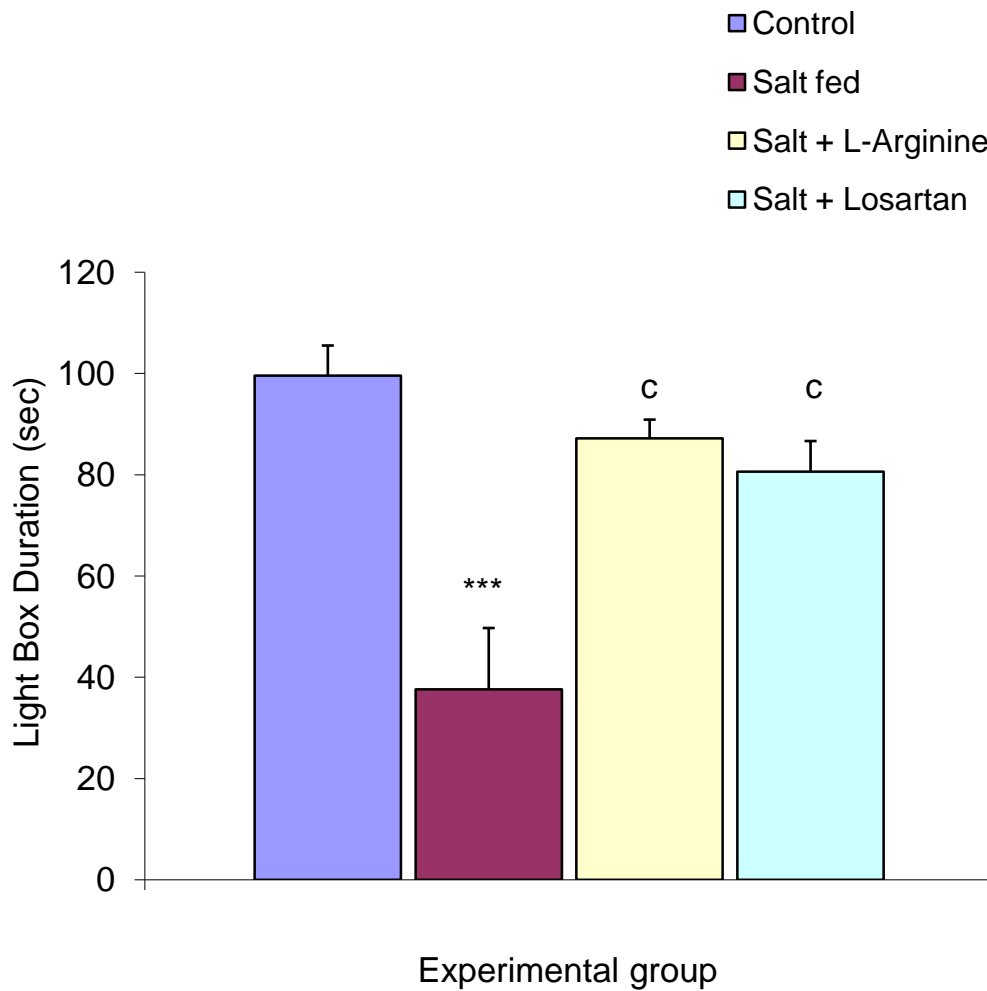


Figure 8: Light box duration in the control and test groups during the light dark transition box test.

Values are expressed as mean +SEM, n = 5.

*** = $p < 0.001$ vs control

c = $p < 0.001$ salt fed

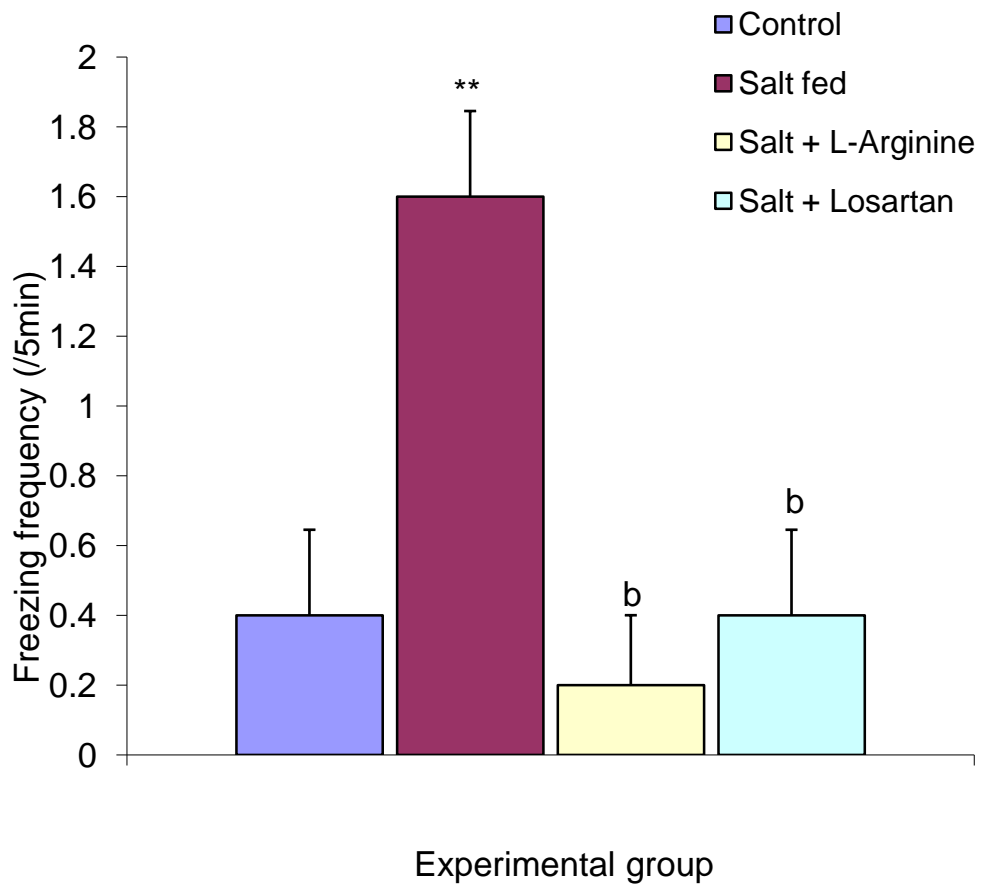


Figure 9: Freezing frequency in the control and test groups during the open field maze test.

Values are expressed as mean +SEM, n = 5.

** = $p < 0.01$ vs control

b = $p < 0.01$ salt fed

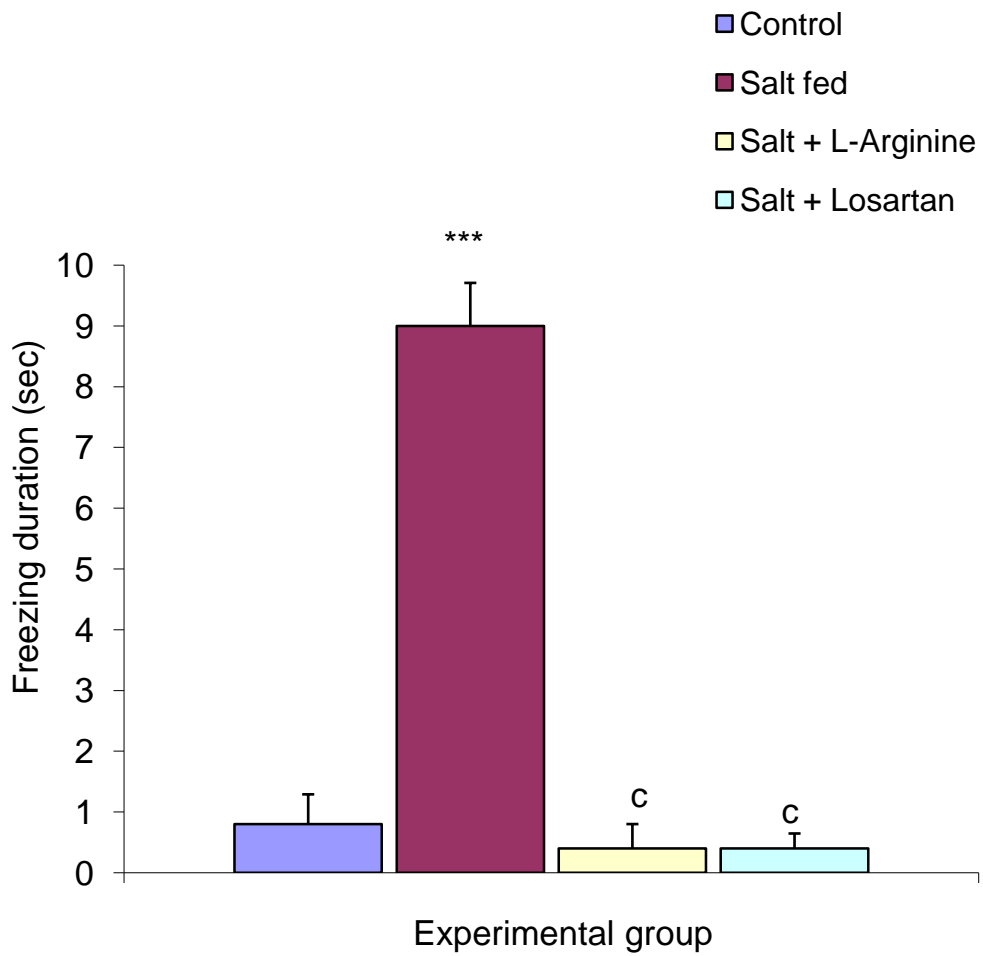


Figure 10: Freezing duration in the control and test groups during the open field maze test.

Values are expressed as mean +SEM, n = 5.

*** = $p < 0.001$ vs control

c = $p < 0.001$ salt fed

DISCUSSION AND CONCLUSION

This study evaluates anxiety and locomotor behaviour in high-salt fed Mice treated with L-arginine and Losartan. High salt intake has been suspected as contributor to many neuropsychiatric illness including; depression and anxiety disorder, reduces locomotor activities^{11,12} demonstrated that excess salt induces anxiety, unusual behaviour and memory impairment in the brain of aging rats. The High energy in synapses, along with increased output of reactive oxygen species is detrimental during increased stress especially in condition such as neurodegenerative disorders like Alzheimer's and Parkinson's diseases¹³.

Anxiety and locomotive behaviours were assessed using Elevated+ Maze (EPM) and Light/Dark transition box (LDTB) test. Anxiety behaviour in the mice such as grooming, freezing, stretch attend posture (SAP), line crossing, head dipping, closed arm activity, duration of stay in light/dark transition box were studied within the experimental environment. The Elevated+ Maze test is an apparatus which consists two "open arms and closed arms" (X). The open arms are aversive to mice because they are open and the maze is elevated¹⁴. The closed arms provide a sense of safety because they are enclosed. Specifically, this research work aimed to evaluate if high salt fed mice treated with L-arginine has any improvement in behaviour during the experiment. Behaviour like transition frequency from open to close arms, the grooming duration, urination and defecation were observed. The light-dark transition box (LDTB) is an unconditioned anxiety behaviour test, it involves exploring an environment while avoiding bright light¹⁵. The box is divided into two compartments, light and dark. Generally the dark compartment is a smaller and is considered "safe" to mice. Behaviour like the number of line crossing, head dipping and frequency, stretch attend posture, etc. were used to measure anxiety

and locomotorbehaviour. A low frequency of thesebehaviour indicates high anxiety level.

In **elevated plus maze**, the frequency of stretch attend posture (SAP), grooming frequency (GF), Grooming duration (GD), Close arm entry (CAE) and Close arm duration (CAD) increased in the salt group. In the L-arginine group, SAP, GF, GD, CAE and CAD were reduced. In the Losartan group, SAP, GF, GD, CAE and CAD were reduced. The EPM test indicates that high salt fed mice is observed with increased anxiety while L-arginine and Losartan showed decreased anxiety and improved behaviour.

In the Light-dark test box (LDTB); SAP, GF, GD and DBD(dark box duration) is increased in the salt group. In light box duration (LBD); line crossing (LC) and transition frequency is **alsoreduced in the salt group than the Control**. In the L-arginine group, LBD and transition frequency is increased while SAP, GF, GD, DBD decrease compared to the salt group. In the Losartan group, LBD and transition frequency is increased while SAP, GF and DBD decreased compared to the salt group. It is observed from LDBT that high salt increased anxiety and reduced locomotor activities while L-arginine and Losartan decreased anxiety and improved locomotorbehaviour.

This study is similar to earlier report¹⁶ which opined that L-arginine helps to reduce anxiety and negative effects of stress, improves cognitive function and exploration in mice^{17,18} revealed that L-arginine improves locomotor activities in rats.

Observations from this work revealed that both L-arginine and Losartan reduces anxiety, as well as improves locomotorbehaviour in mice.

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