Ameliorative Potential of Aminophylline In Restrain Stress Induced Behavioural and Biochemical Alterations

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Abstract This study is designed to investigate the attenuating prospective of aminophylline in immobilization stress generated behavioural changes in rats. Animals were exposed to restrain stress before being subjected to varying doses of aminophylline (1mg/kg, 2mg/kg and 4mg/kg). Behavioural changes were analyzed to assess the intensity and the degree of the stress, by estimating the changes in the exploratory behaviour, spontaneous activity and social behaviour using various paradigms. As a consequence of stress, the behavioral patterns so changed were assessed in the terms of changes in the locomotor activity, number of head dips and increased avoidance behaviour. Aminophylline (4mg/kg) modulated the stress produced changes in the behaviour and oxidative stress generated biochemical alterations in a significant manner (p<0.001). The results so obtained suggest that upon exposure to stress, animal behavioural patterns, biochemical markers levels changed and these changes wereefficiently modulated by aminophylline at therapeutic doses.

Keywords: Aminophylline, Behavioural alterations, Biochemical alterations, Restraint stress.

1. INTRODUCTION

Stress has a complex neurobiology which involves release of stressor hormones (adrenocorticotrophin releasing hormone (ACTH), Nor-adrenaline (NA)) which further mediate the stress responses. Chronic stress leads to depression. In Depression, there is a release of mediators which are pro-inflammatory in nature, mainly cytokinesTNF-α, IL-1, IL-6 and IFN-γ) in

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brain, which consequently provoke the HPA- axis. Triggering of the HPA-axis, causes the release of Adrenocoticotrophin hormone, which manifests the changes in the behaviour as a response to stress(Miller *et al.* 2009). Release of NA and over activity of α and β receptors in brain, another effect produced in response to stress promotes the levels of cytokines in brain(Johnson *et al.* 2005).

It has been reported that stressful conditions result in the activation of TNF-α in brain. TNF-α being a neuro-inflammatory mediator (Alleva *et al.*1993, Calamandrei *et al.* 1991) triggers a neuro-inflammatory cascade which results in enhanced expression of cyclo-oxygenase-2 (COX-2) enzyme (Nogawa *et al.*1997). Increased levels of COX-2 results in production of prostaglandin, PGE_2, which promotes febrile and behavioral alterations (Vesce *et al.* 2007).

Aminophylline a competitive non selective phosphodiesterase inhibitor which has been remarkably used in the treatment of acute and chronic bronchial asthma, apnea syndromes, COPD, in treatment of bradyarrythmias in elderly (Vassallo and Lipsky, 1998). Another astonishing use of theophylline is the induction of the apoptosis in the chronic lymphocytic leukemia cells along with chlorambucil. Recently, aminophylline has been investigated as a potential adjunct along with furosemide in increasing urine output in fluid overloaded patients.

The activity of aminophylline is due to its metabolic product, theophylline. Theophylline has multiple mechanism of action which includes phosphodiesterase enzyme inhibition, adenosine receptor antagonism and immunomodulatory effects(Vassallo and Lipsky, 1998). The anti-inflammatory activity is attributed to modulation of cytokine production. Exposure to theophylline, triggers an increase in the levels of IL-10, an anti-inflammatory cytokine, which exerts an inhibitory control over the production of the pro-inflammatory cytokines (IL-2, IFN-γ, IL-5, TNF-α and IL-8).Thus it can be proposed that the compounds which either promote the production of anti-inflammatory cytokines or the one blocking the inflammatory effects of pro-inflammatory cytokines can be a potential target for modulation of restrain stress generated oxidative stress mediated inflammation and the resultant behavioural alterations. However, the anti-stress potential of aminophylline is not yet investigated, despite its significant potential to inhibit TNF-α. Thus, this study focuses on the investigation of the attenuating potential of aminophylline in restrain stress generated behavioral alterations in rats. The alterations of reduced glutathione, lipid peroxidase, catalase and superoxide dismutase in the restrain stress induced animals was also studied to determine the biochemical basis of the anti-stress mechanism of aminophylline.

2. MATERIALS AND METHODS

2.1 Animals

Sprague Dawley rats of female sex, in frank oestrus stage, (Kaur *et al.* 2010, Manchanda *et al.* 2011,) weighing 150-250 g, were used inthis study. Animals were fed on standard laboratory diet and animals were housed in the Departmental animal house, Chitkara college of Pharmacy, Chitkara University, Punjab. They were exposed to natural cycles of light and dark. Care of the animals was carriedout as per the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) and the experimental protocol was approved by the Institutional Animal Ethics Committee.

2.2 Drugs and Chemicals

Aminophylline, used in the study, was available as (anhydrous) aminophylline from AartiIndustries Limited, Jalandhar, India. Other chemicals employed in the study were of analytical grade and purchased from Sigma Aldrich, U.S.A and SDFCL (SD-Fine Chemical Limited, Mumbai).

2.3 Experimental Procedure

2.3.1 *Induction of Restrain Stress*

A wire mesh restrainer was used to induce restrain stress. Dimensions of the restrainer were 11 cm × 8 cm × 8 cm and the animals were subjected to immobilization for 3.5 hours (Madhyastha *et al.* 2008). The restrainer had a wooden base and stainless steel wire mesh hinged to the base. The restrainer restricts the movement of the animals without producing any sort of discomfort and pain.

2.4 Behavioral Measurements

Prior Acclimatization of rats was done on each test, 5 min daily for 3 days. The rats were then subjected to restrain stress, and then chain of behavioral tests was performed in animals beginning with the estimation of index of curiosity to the social behaviour of the animal with a gap of 5 min between each test. The experimental protocol was carried out in early morning as the corticosterone levels are at peak in the morning. The behavioural models so employed in the study were cleaned using alcohol.

2.4.1 *Hole board test*

This test is used for the measurement of the curiosity of the animal to explore the surroundings. Hole board is made up of a wooden box of the dimensions

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68 cm × 68 cm with the walls 40 cm high. The box was placed on a stand at a height of 28 cm from the ground. The holes (4 in number of diameter 4 cm) were 28 cm away from the corners of the box and using tape the floor of the box was divided into one central and four peripheral areas. Tape was used to mark the boundaries into four peripheral areas and one mid area. The mid area was marked by tape from the four sides in such a way that the distance between the central area and the walls is 20 cm and the peripheral areas are marked diagonally. The four holes lie at the corners of the central area. Behavioural parameters measured in hole board are number of head dips and the incidence of rearing(Brown and Nemes, 2008).

2.4.2 *Open field test*

This test is used to assess the alertness and index of curiosity. The apparatus used for the open field test is made up of wooden box measured 90 cm × 90 cm × 38 cm. The walls are darkened using black paintand the floor is painted white (Blokland *et al.* 2002; Roman *et al.* 2006). Floor of the box was partitioned into 16 marginal and 9 central small squares(in total there were 25 squares) of dimensions 17 cm × 17 cm. Number of line crossing and the time spent in the peripheral and central areas were measured by placing the animal in the centre of the wooden box for 10 min.

2.4.3 *Social interaction test*

Social interaction test is done in the open field box. Time of avoidance and following are noted in seconds for an interval of 10 min by placing the animals in the center of the box (Rex *et al.* 2004; Toth *et al.*, 2008). Nonsocial behavior(Aggressive behavior such as boxing, biting or threatening the partner rat apart from self-grooming/ignoring the partner) and social behaviour(following other rats) are well characterized during assessment of 10 min.

2.5. Biochemical Estimations

2.5.1 *Tissue Homogenate preparation*

Biochemical analysis was done using brain tissue homogenate, which was prepared by removing the brain from the sacrificed animal. After the behavioural parameters assessment, animals were decapitated and the brain was removed and a tissue homogenate of 10% (w/v) in 0.1 M phosphate buffer (pH 7.4). This homogenate was then centrifuged for 15 min at a speed of 10,000 rpm. The clear supernatant so obtained is employed for different biochemical estimations(Kumar *et al.*, 2009).

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2.5.2 *Estimation of Reduced Glutathione*

Reduced Glutathione (GSH) is a powerful antioxidant. Immobilization stress results in generation of oxidative stress and consequently, free radicals are also generated. Glutathione is capable of scavenging Reactive Oxygen Species (Harris, 1992).Homogenate is mixed with equal volume of 20% tricholoroacteic acid (TBA) which contains 1 mM EDTA, which causes precipitation of tissue proteins. Then this mixture is subjected to centrifugation at 200 rpm for 10 min. To this 200 μl of the supernatant, 1.8 ml of Ellman's reagent(5, 5'□dithio *bis*2-nitrobenzoic acid- 0.01 M prepared in 0.3 M phosphate buffer with 1% of sodium citrate soluon). The volume in the test tubes is then made up to 2ml. The mixture is then analysed`at UV spectrophotometer at 412 nm against blank and the result is expressed in micromole/g brain. The absorbance values of the test sample were compared with the standard curve of GSH(Ellman, 1959).

2.5.3 *Estimation of lipid peroxidase*

Oxidative stress, generated as a result of restrains stress results in lipid peroxidation and cell damage. Peroxidation product, Thiobarbituric Acid Reacting Substances (TBARS), serves as a specific indicator (Das *et al.* 2000). For the measurement of the TBARS 0.5 ml of tissue homogenateis mixed with 0.5 ml tris HCL (pH 7.4). The above mixture is then incubated at 37°C for 2 hours, and then 1ml 1 ml of ice cold 10% TCA is added. Centrifugation of the mixture is done for 10 min at 1000rpm speed. The supernatant so obtained is collected and to this 1ml of 0.67% TBA is added. The above mixture is then heated for 10 min in a water bath and cooled. Finally, 1ml of distilled water is added and then optical density is measured taking distilled water as blank at 532 nm (Wills, 1996).

2.5.4 *Estimation of catalase*

Another endogenous antioxidant that catalyses the dismutation of hydrogen peroxide into water and oxygen is a haem-containing enzyme called as catalase. It decreases the level of free radicals which are generated due to restrain stress (Deisseroth and Dounce, 1970). For the estimation of the catalase, 3mL of H_2O_2in phosphate buffer is added to 0.05 ml of the homogenate and the absorbance is measured at 240 nm. Results are expressed as micromole H_2O_2 decomposed per mg of protein/min (Luck, 1971).

2.5.5 *Estimation of superoxide dismutase*

Superoxide Dismutase (SOD) isan important endogenous antioxidant enzyme and catalyzes the dismutation of the highly reactive superoxide anion to oxygen and to the less reactive species hydrogen peroxide(Fridovich, 1983; Willcox

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et al. 2004). To estimate superoxide dismutase, 2ml of NBT (Nitrobluetet-razolium) is added to 0.5 ml hydroxylamine HCl and then 0.1ml of homogenate is added. Change in the absorbance was recorded using distilled water as blank at 560 nm for 2 min at 30/60 sec intervals.

2.6 Experimental Protocol

Seven groups, comprising five Sprague Dawley female rats were employed in the present study.

Group I: *Normal control*

The rats are not given any type of stressors, behavioural parameters were noted. A sequence was set up for the behavioural assessment starting with the hole board followed by the open field test and social interaction test. After the behavioural assessment, tissue homogenates were prepared for biochemical analysis by sacrificing the animals

Group II: *Stress control group*

In this group, the animals were exposed to restrain stress for three and half hours. Thereafter, behavioural parameters were estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

Group III: *Aminophylline (1 mg/kg) + stress control group*

In this group, the animals were pretreated with Aminophylline (1 mg/kg i.p.) 30 min prior to exposure to stress. The animals were then exposed to stress (3.5 hours). Thereafter, behavioural parameterswere estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

Group IV: *Aminophylline (2 mg/kg) + stress control group*

In this group, the animals were pretreated with Aminophylline (2 mg/kg i.p.) 30 min prior to exposure to stress. The animals were then exposed to stress (3.5 hours). Thereafter, behavioural parameters were estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

Group V: *Aminophylline (4mg/kg) + stress control group*

In this group, the animals were pretreated with Aminophylline (4 mg/kg i.p.) 30 min prior to exposure to stress. The animals were then exposed to stress

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(3.5 hours). Thereafter, behavioural parameters were estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

Group VI: *Aminophylline (4 mg/kg per se)*

In this group, the animals were not exposed to restrain stress, but aminophylline (4 mg/kg i.p.) was administerd to the rats to assess the per se effect of aminophylline. Thereafter, behavioural parameterswere estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

Group VII: *Diazepam (2mg/kg) + stress control group*

In this group, the animals were pretreated with Diazepam (2 mg/kg i.p.) 30 min prior to exposure to stress. The animals were then exposed to stress (3.5 hours). Thereafter, behavioural parameters were estimated in a sequential manner. Then the tissue homogenates were prepared for analyzing various biochemical parameters.

2.7. Statistical Analysis

The results were expressed as Mean $\pm$ Standard Error of Means (S.E.M.). The results were analyzed using one-way ANOVA followed by post-hoc analysis using Tukey's Multiple Comparison Test for comparison between different groups. The p value $<0.0001, <0.01, <0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Consequence of immobilization stress and aminophylline on exploratory behaviour of rats(head dips and rearing) in Hole board test

Index of curiosity or exploration, in hole board test is analyzed by number of head dips and the frequency of rearing. The rats subjected to restrain stress, had a decreased exploratory behavior as indexed by the number of head dips and rearing in comparison to the normal control group. Pretreatment with aminophylline (1mg/kg, 2mg/kg, and 4mg/kg i.p.), however had a modulating effect on the behavior of the animal as depicted by a significant increase in the number o head dips and rearing as compared to the stress control group. Aminophylline (4mg/kg i.p) had a significant attenuating effect on the behavioural alteration produced by restrain stress which was comparable to the standard drug **(Figure 1 and 2)**.
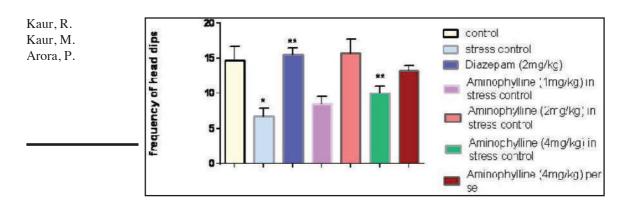
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Figure 1: Effect of restrain stress and aminophylline on frequency of head dips in the hole board test. Values are expressed as mean $\pm$ S.E.M **P<0.001 as compared to normal control group, ***P<0.001 as compared to stress control group, **P<0.01 as compared to stress control group, *P<0.05 as compared to diazepam in stress control group.
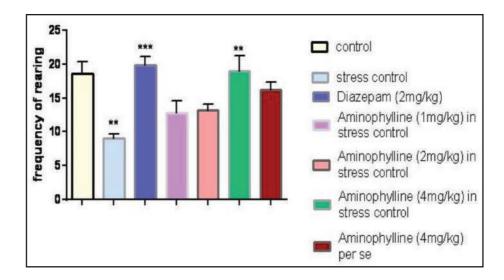

Figure 2: Effect of restrain stress and aminophylline on frequency of rearing in the hole board test. Values are expressed as mean $\pm$ S.E.M **P<0.01 as compared to normal control group, ***P<0.001 as compared to stress control group, *P<0.05 as compared to diazepam, *P<0.05 as compared to stress control group.
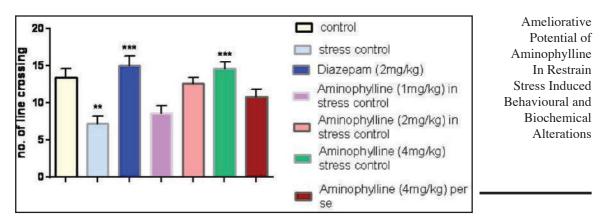
Figure 3: Effect of restrain stress and aminophylline on frequency no. of line crossing in open field test. Values are expressed as mean $\pm$ S.E.M **$P<0.01$ as compared to normal control group, ***$P<0.001$ as compared to stress control group, ** $P<0.01$ as compared to stress control group, ***$P< 0.001$ as compared to diazepam in stress control group.

Table 1: Effect of restrain stress and aminophylline on line crossing and rearing on open field test. Statistically analyzed by one-way analysis of variance (ANOVA) followed by post-hoc analysis using Tukey's Multiple Comparison Test. Values are expressed as Mean $\pm$ S.E.M with n=5 in each group. **$P<0.01$ as compared to normal control group, ***$P<0.001$ as compared to stress control group, **$P<0.01$ as compared to stress control group, *$P<0.05$ as compared to diazepam in stress control group.

Experimental Groups	Line crossing	Rearing
Normal control	13.4 ± 1.2	12 ± 1.67
Stress control	7.2 ± 1.0**	5.50 ± 1.00**
Diazepam (2mg/kg)	15.0 ± 1.30***	1.3 ± 1.1***
Aminophylline (1mg/kg) in stress control group	8.6 ± 1.0**	7.0 ± 0.11
Aminophylline (2mg/kg) in stress control group	12.6 ± 0.8	10 ± 1
Aminophylline (4mg/kg) in stress control group	14.6 ± 0.9***	12.15 ± 1.67**
Aminophylline (4mg/kg) per se	10.8 ± 10	13.25 ± 0.68

3.2 Effect of immobilization stress and aminophylline on spontaneous activity and exploratory behaviour) in open field test:

Motor activity and exploration are articulated as number of line crossing and the frequency of rearing. The rats subjected to restrain stress, had a decreased

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exploratory behavioras expressed by the number of rearing in comparison to the normal control group. The index of wakefulness is indicated by the motor activity, which significantly decreases in the restrain stress group as compared to the group of animals not subjected to stress(normal control group). Pretreatment with aminophylline (1mg/kg, 2mg/kg, and 4mg/kg i.p.), however had a modulating effect on the behavior of the animal as depicted by a significant increase in the number of rearing and line crossing as compared to the stress control group. Aminophylline (4mg/kg i.p) had a significant attenuating effect on the behavioural alteration produced by restrain stress which was comparable to the standard drug(**Figure 3 and 4, Table 1**).

3.3 Effect of immobilization stress and aminophylline on rats in social interaction test:

The social behaviour and the non- social behaviour is analyzed by determining the average time of following and avoidance in the social interaction test. In case of pretreatment with aminophylline (1mg/kg, 2mg/kg and 4mg/kg i.p) protocols, time of following the partner (social behavior) was primarily shown, while in the restrain stress group time of avoidance was shown mainly (Table 2).
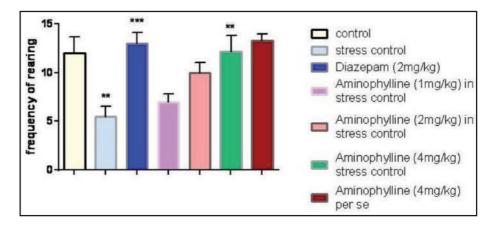

Figure 4: Effect of restrain stress and aminophylline on frequency no. of rearing in open field test. Values are expressed as mean $\pm$ S.E.M ** P<0.01 as compared to normal control group, *** P<0.001 as compared to stress control group, * P<0.05 as compared to stress control group, * P< 0.05 as compared to diazepam in stress control group.
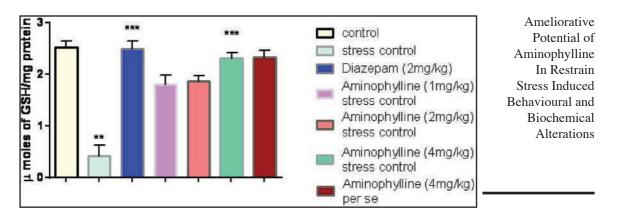
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Figure 5: Effect of restrain stress and aminophylline on changes in reduced glutathione levels (GSH). Results are represented as the mean $\pm$ S.E.M. with n = 5 in each group, *P< 0.05 as compared to normal control group.

Table 2: Effect of restrain stress and aminophylline on the time of following and avoidance (s) in the social interaction test. Values are expressed as Mean $\pm$ S.E.M with n=5 in each group. **P<0.01 as compared to normal control group, *P<0.05 as compared to stress control group, *P<0.05 as compared to diazepam in stress control group.

Experimental Groups	Time of following (s)	Time of avoidance (s)
Normal control	3.83 ± 0.79	5 ± 1.23
Stress control	4.5 ± 0.76*	6 ± 0.81*
Diazepam (2mg/kg)	3.5 ± 0.670*	$7.5 \pm .118$*
Aminophylline (1mg/kg) in stress control	3.33 ± 0.61	7.66 ± 0.802
Aminophylline (2mg/kg) in stress control	3.5 ± 0.288	5 ± 1
Aminophylline (4mg/kg) in stress control	1.33 ± 0.49**	8 ± 0.516**
Aminophylline (4mg/kg) per se	1.5 ± 0.670	3.5 ± 0.763

3.4 Alterations in the reduced glutathione in immobilization stress and pretreatment with aminophylline groups.

Reduced glutathione levels decreased as a consequence of immobilizationstress in contrast to the normal control group. Pretreatment with aminophylline (1 mg/kg, 2mg/kg and 4mg/kg i.p.) increased the brain Reduced glutathione levels in a dose dependent manner. **(Figure 5 and Table 3).**

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3.5 Alterations in the Lipid Peroxidation (LPO) level due to restrain stress and aminophylline

Thiobarbituric acid reacting substances (TBARS) levels, was increased, in the brain of the rats subjected to restrain stress when compared with the normal control group. Aminophylline (1mg/kg, 2mg/kg and 4mg/kg i.p.), in a dose dependant manner, has decreased the brain TBARS levels **(Figure 6 and Table 3)**.
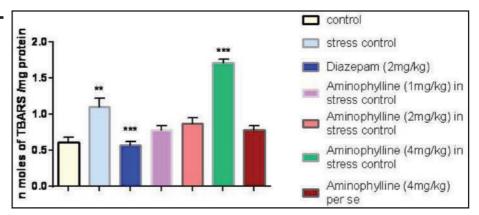

Figure 6: Effect of restrain stress and aminophylline on changes in Lipid Peroxidation (LPO) levels. Results are represented as the mean $\pm$ S.E.M. with n = 5 in each group, **$P<0.01$ as compared to normal control group, *** $P<0.001$ as compared to stress control group, ** $P<0.01$ as compared to diazepam.
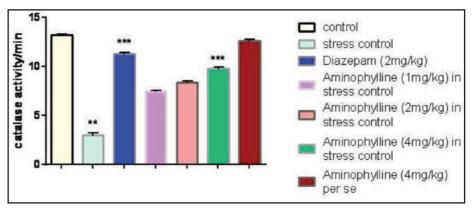

Figure 7: Effect of restrain stress and aminophylline on changes in catalase (CAT) levels. Results are represented as the mean $\pm$ S.E.M. with n = 5 in each group, ****$P<0.0001$ as compared to normal control group, **** $P<0.0001$ as compared to stress control group, **** $P<0.0001$ as compared to stress control group, ****$P<0.0001$ as compared to diazepam.

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3.6 Changes in Catalase levels due to immobilization stress and aminophylline

Decreased brain catalase levels wereobserved in restrain stress. Aminophylline protocols (1mg/kg, 2mg/kg and 4mg/kg i.p.), dose dependently, significantly elevated the brain catalase levels **(Figure 7 and Table 3).**

Table 3: Effect of restrain stress and aminophylline on changes in biochemical parameters such as Lipid Peroxidation (LPO), Superoxide Dismutase (SOD), Reduced Glutathione (GSH) and Catalase (CAT). Statistically analyzed by One-Way Analysis of Variance (ANOVA) followed by post-hoc analysis using Tukey's Multiple Comparison Test. Values are expressed as Mean $\pm$ S.E.M. with n= 5 in each group, $**p<0.01$, $****p<0.0001$, $*P<0.05$ as compared to normal control group and $***p<0.001$, $**P<0.01$ as compared to stress control group.

Experimental Groups	LPO (nmol/mg protein)	SOD (µmol/mg protein)	CAT (µmol/mg protein)	GSH (µmol/mg protein)
Normal control	0.61 ± 0.07	2.63 ± 0.12	13.22 ± 0.05	2.52 ± 0.12
Stress control	$1.09 \pm 0.12**$	$0.51 \pm 0.21**$	$2.97 \pm 0.2**$	$0.41 \pm 0.21**$
Diazepam (2mg/kg)	$5.70 \pm 0.50***$	$2.49 \pm 0.16***$	$11.24 \pm 0.16***$	$2.49 \pm 0.16***$
Aminophylline (1mg/kg) in stress control	1.06 ± 0.08	1.81 ± 0.14	7.43 ± 42.53	1.8 ± 0.144
Aminophylline (2mg/kg) in stress control	0.86 ± 0.08	1.96 ± 0.10	8.39 ± 0.10	2.31 ± 0.11
Aminophylline (4mg/kg) in stress control	$1.70 \pm 0.50***$	$2.21 \pm 0.11***$	$9.78 \pm 0.11***$	$2.33 \pm 0.14***$
Aminophylline (4mg/kg) *per se*	0.78 ± 0.6	2.58 ± 0.14	12.65 ± 0.14	158.8 ± 0.14

3.7 Alterations in the Superoxide Dismutase (SOD) level due to restrain stress and aminophylline:

Decreasedbrain superoxide dismutase (SOD) levels were observed in rats after exposure to restrain stress. Aminophylline protocols (1mg/kg, 2mg/kg and 4mg/kg i.p.), increased SOD levels significantly **(Figure 8 and Table 3).**
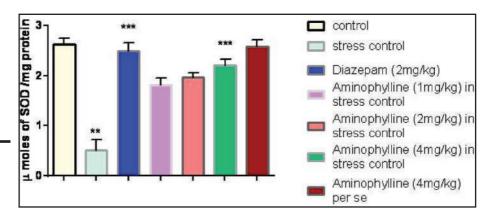
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Figure 8: Effect of restrain stress and aminophylline on changes in Superoxide dismutase (SOD) levels. Results are represented as the mean ± S.E.M. with n = 5 in each group, ****P<0.001 as compared to normal control group, ****P<0.001 as compared to stress control group, *P<0.05 as compared to diazepam, ****P<0.01 as compared to stress control group.

4. DISCUSSION

Aminophylline, being a compound of theophylline and ethylene diamine (85:15) gets converted into theophylline in-vivo (Abe *et al.* 2008). Theophylline is a inhibitor of phosphodiesterase enzyme andcauses an increase in intracellular cAMP which in turn activates protein kinase (pKA) and inhibits TNF-α mediated leukotriene synthesis suppressing further inflammation (Barnes, 1998).

TNF-α is a neuroinflammatory mediator (Nogawa *et al.* 1997; Vesce *et al.* 2007) which triggers a cascade of events resulting in provoking the expression of cyclo-oxygenase-2 (COX-2) enzyme (Nogawa *et al.* 1997). Increased COX-2 expression results in production of prostaglandin, PGE_2, which promotes febrile and behavioral alterations (Hayley *et al.* 2008). There are a number of publications which provide the evidence for presence of TNF-α in the brain of rodents as well as humans under normal and pathological conditions (Olmos and Llado, 2014). Aminophylline, a competitive non-selective phosphodiesterase inhibitor significantly attenuated($p<0.001$, 4mg/kg i.p.) the restrain stress induced behavioral alterations suggesting that pharmacological modulation of TNF-α may be one of the factors for counteracting the stress associated changes in the behaviour. From the above results so obtained, the significant levels of amelioration can be observed with the dose of aminophylline (4mg/kg i.p.; $p<0.001$) as compared to the other two doses (1 mg/kg, 2mg/kg).

In this study, it can be seen that restraining of female rats for three and half hours resulted in significant behavioral alterations which includes decreased

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spontaneous activity, exploratory behaviour and an increased non-social behaviour. The different behavioural parameters are analyzed using various paradigms such as hole board, open field and the social interaction test. Furthermore, to establish the biochemical basis of stress various biochemical parameters such as reduced glutathione, TBARS, SOD and catalase are also estimated, thereby, suggesting that restrain stress induces oxidative stress that leads to neurodegenerative effects.

Therefore, it may be concluded that during stressful conditions, TNF-α expression increases and along with other neuroinflammatory mediators, is responsible for behavioral alterations and aminophylline modulates the TNF-α activity in brain thereby resulting ameliorative potential against restrain stress induced behavioral alterations.

5. CONCLUSION

From the present study, it may be proposed that aminophylline modulates restrain stress induced behavioural and biochemical effects and it may be possibly be attributed to alter the TNF-α expression during immobilization stress. The above findings from the study, suggests that aminophylline may be further investigated as a potential anti-anxiety and anti-stress agent.

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