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DEFENSIVE ROLE OF DIAZEPAM AGAINST SLEEP DEPRIVED BEHAVIOUR

Biochemistry

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ABSTRACT

The aim of this study was to explore the defensive role diazepam against stress produced as a result of sleep deprivation in albino mice. So, in this study healthy male albino mice weighing between 25-30 grams were divided into three groups with six animals in each group. The first group A were represented as negative control, i.e. (without sleep deprivation), second group B were represented as positive control and subjected to induce 48 hours of sleep deprivation (by placing on a grid suspended over water, based on modified method of Shinomiya *et al*.) Whereas, the study group C was sleep deprived and administered diazepam to strength of 0.02mg/ml, by dissolving 5mg tab in 250ml of gum acacia orally once daily for 24 days. The defensive role of diazepam was evaluated by measuring anxiety level or stress produced as a result of sleep deprivation by Elevated plus maze model.

KEYWORDS

Sleep deprivation, diazepam, anxiety level, albino mice, Elevated plus maze model.

INTRODUCTION:-

Scientists recommend approximately eight hours of sleep a night to promote efficient performance and thinking. Sleep is regulated by several basic mechanisms, and when these systems disrupted sleep deprivation occur. An inability to sleep is a source of distress[1], which leads to increase in anxiety level[2], and behavioral alteration[3].The disturbance of normal sleep has been reported to produce harmful effect to metabolic as well as endocrine functions of the body.[4,5] Sleep deprivation is becoming increasingly common in today's society, because of busy life style.Individuals with sleep deprivation most often report a combination of difficulty falling asleep and intermittent wakefulness during sleep.[6,7] More and more people are becoming victims of the consequences of sleep deprivation, which affects both physical as well as mental health.[8]There are various causes of sleep deprivation. Among these, Shift work[9], poor sleep hygiene such as drinking coffee or smoking cigarettes close to bedtime[10], certain sleep disorders like obstructive sleep apnea[11], snoring, periodic limb movement[12] etc are common. Nowadays, excessive uses and sort of addiction to mobile phone, uses of computer and video game before bed time, especially in teenagers, lead to sleep deprivation, that has been shown to negatively affect many physiological, cognitive, and behavioural measures within the body.[13,14] That is why this study was performed to increase the awareness among general population for better understanding of the consequences of sleep deprivation.

Diazepam, a classical Benzodiazepines, often referred to as sedatives,a class of drug used primarily in the treatment of various disorders like anxiety, insomnia, muscle relaxant, epilepsy and anaesthesia.[15] Although, Diazepam is non selective $GABA_A$ receptor agonist[16], but it is potent anxiolytic drug by modulating GABAergic function through α_2GABA_A[17] receptors . Moreover, its high therapeutic index and low toxicity has led to choose the drug for the above study.

MATERIALS AND METHOD:-

The present work was conducted in the Postgraduate Laboratory of the Department of Pharmacology and Therapeutics of tertiary care centre after ethical approval from the Institutional Animal Ethics Committee (IAEC) Guidelines.

Experimental Animals:-

In this experiment a total of 18 apparently healthy male albino mice weighing between 25-30 grams were used. The animals were kept at controlled laboratory conditions (22±2°C, 55±5% RH, and equal dark-light cycle, acclimatization period: 1 week).

Chemicals and Reagent kits:-

Diazepam, Distilled water, Normal Saline, Gavage tube, 1% gum acacia suspension.

Dose of the Drugs:-[18]

Dose of the drugs will be calculated from the standard clinical human dose on the basis of surface area. Surface area ratio of 20g mice for 70 kg man is 0.0026. Thus human dose of any drug (for a 70 kg person) multiplied by 0.0026 gives the value of that drug for 20g of mice.

Sleep deprivation protocol:-

Animals were sleep deprived for 48 hrs by placing on a grid suspended over water, based on modified method of Shinomiya *et al*.[19] In this method animals were placed on a grid floor(29*15*7cm) inside the plastic cage filled with water to 1cm below the grid surface for 48 hours. The stainless steel rods of the grid (3mm) will be set 2cm apart from each other. Food and water will be provided *ad libitum*.

Experimental outline:-

The animals under study were classified into following groups randomly selecting 6 mice in each group.

GROUP A=Normal (Negative Control)
GROUP B=Sleep Deprived (Positive Control)
GROUP C= Sleep Deprived mice treated with Diazepam.

After allowing 48 hours of sleep deprivation, the stress produced as a result sleep deprivation was measured by **elevated plus maze model,** which consists of two open arms (16*5cm) and two closed arms (16*5cm) and is placed at a height of 25cm. The animals were placed individually at the center of the maze with head facing the open arm. During the 5 minute test, the number of entries into the open and closed arms and the time spent in each arm were recorded. The assessment was performed on day 0, 6th, and 24th day. All the treatments were carried out for a period of 24 days.

STATISTICAL ANALYSIS:-

Statistical analysis of data was carried out by employing analysis of variance (Snedecor and Cochran, 1967). One way ANOVA test was used to compare the effect of drugs on different group. Tukey's HSD test was used for post-hoc analysis of significant overall differences.

RESULTS:-

Table:-1 Showing changes in no. of entries in open arm in elevated plus maze model on 0, 6th, and 24th day between Group A and B.

DAY	Group A	Group B	Difference in Mean	Significance
0 DAY	2.67±0.516	3.50±0.548	0.833	0.123*
6th DAY	3.50±1.049	1.33±0.516	2.167	0.001**
24th DAY	3.33±1.211	1.00±0.000	2.333	0.002**

****P<0.05 -Significant and *P>0.05- Non significant**

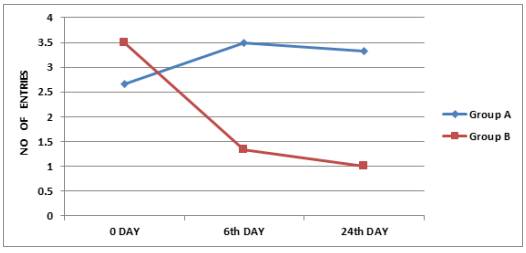

Figure:-1 Shows Comparison graph of no. of entries in open arm in elevated plus maze model on 0, 6th and 24th day between Group A&B.

Table:-2 Showing changes in time spent (sec) in open arm in elevated plus maze model on 0, 6th, and 24th day between Group A and B.

DAY	Group A	Group B	Difference in Mean	Significance
0 DAY	54.00±2.828	56.83±4.355	2.833	0.759*
6th DAY	52.17±3.971	15.67±1.633	36.500	0.000***
24th DAY	54.17±2.787	12.67±1.751	41.500	0.000***

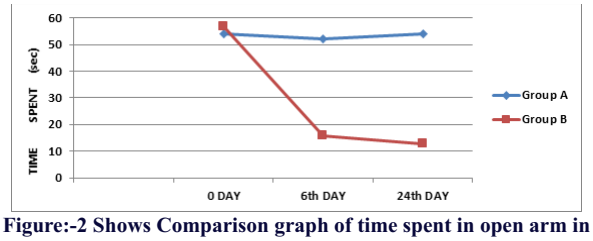

Figure:-2 Shows Comparison graph of time spent in open arm in elevated plus maze model between Group A&B.

Table:-3 Showing changes in no. of entries in open arm in elevated plus maze model on 0, 6th, and 24th day between Group B&C

DAY	Group B	Group C	Difference in Mean	Significance
0 DAY	3.50±0.548	3.50±0.548	0.000	1.000*
6th DAY	1.33±0.516	7.50±1.049	6.167	0.000***
24th DAY	1.00±0.000	5.83±1.472	4.833	0.000***

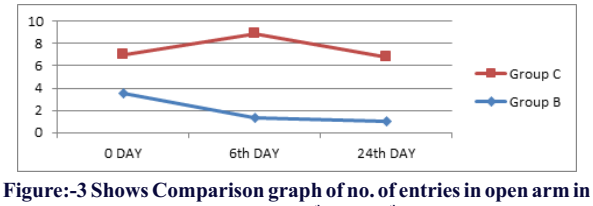

Figure:-3 Shows Comparison graph of no. of entries in open arm in elevated plus maze model on 0, 6th and 24th day between Group B&C

Table:-4 Showing changes in time spent in open arm in elevated plus maze model on 0, 6th, and 24th day between Group B&C

DAYS	Group B	Group C	Difference in Mean	Significance
0 DAY	56.83±4.355	57.17±2.787	0.333	1.000*
6th DAY	15.67±1.633	154.17±6.646	138.500	0.000***
24th DAY	12.67±1.751	137.83±6.765	125.167	0.000***

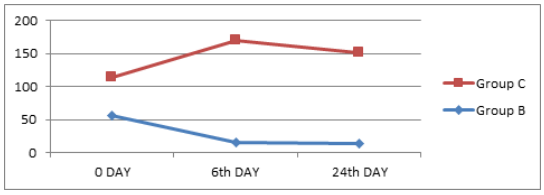

Figure:-4 Shows Comparison graph of time spent (sec) in open arm in elevated plus maze model between Group B&C.

DISCUSSION:-

About 48 hours of sleep deprivation caused severe anxiety in mice which is clear from **Table 1 & 2** that there was decreased in number of entries (62 and 71.42 percent reduction on 6th & 24th day respectively) as well as duration (72.42 and77.70 percent reduction on 6th & 24th day respectively) in open arm of the elevated plus maze model throughout the study, which was statistically significant as compared to group A. This signifies that Stress produced as a result of sleep deprivation causes increase in anxiety level,[20] irritability and poor performance.[21] **Table 3 & 4** shows that pretreatment with diazepam increased number of entries (114.28 and 66.57 percent on 6th & 24th day respectively) as well as duration (169.66 and 141.08 percent on 6th & 24th day respectively) in open arm of the elevated plus maze model throughout the study, which was statistically significant as compared to group B. This is because diazepam is the most potent anxiolytic drug. From **figure 3 & 4**, the graph clearly indicates that the overall influence of diazepam on the number of open arm entries as well as duration had reached statistical significance. This shows the defensive role of diazepam against sleep deprived behaviour.

CONCLUSION:-

From the above study it has been concluded that diazepam is the potent anxiolytic drug, and can be said to have defensive role against sleep deprived behaviour. Also, based on the above findings, more clinical trials can be designed in the future to explore the therapeutic uses of the drug.

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