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Comparative Study of Muscle Relaxant Activity of Alprazolam With Diazepam In Mice

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Abstract:
Background: *A muscle relaxant is a drug which affects skeletal muscle function and decreases the muscle tone. It may be used to alleviate symptoms such as muscle spasm, pain and hyperreflexia. Skeletal muscle relaxants are heterogeneous group of medications that refer to 2 major therapeutic groups: neuromuscular blockers and spasmolytics.* ***Aim and objectives:*** *The study was designed to assess the muscle relaxant activity of alprazolam and compare the muscle relaxant activity of alprazolam with diazepam using albino male mouse as experimental model* ***Materials and methods:*** *The white albino mice were selected, weighed and numbered. A total of 24 animals weighing about 30 grams were selected for the present study. They were divided into four groups each group consists of 6 mice. The animal was placed one by one on the rotarod more than one mouse at a time were placed. Initially free fall off reading was taken before administration of drugs. The free fall off time was noted when the mouse falls from the rotating rod. A normal untreated mouse generally falls off within 3-5 minutes. The reaction time was recorded after 30 minutes interval following administration of diazepam (2mg/kg) and Alprazolam (2mg/kg) administered separately through intraperitoneal mode of inoculation. Then reaction time was also recorded for 3mg/kg and 4mg/kg of Diazepam and alprazolam.* ***Statistical analysis:*** *Statistical analysis done in MS office Excel (2007) by applying one way ANOVA (Data analysis) and calculated the significance of drug. P value <0.05 indicates significant.* ***Results:*** *The study was carried out in albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of diazepam, and alprazolam. On inter drug comparison of the two drugs it was found that, even though alprazolam has slightly higher muscle relaxant effect at increased concentration i.e at 4 mg/kg body weight as compared to drug diazepam, this result is statistically not significant. At decreased concentration i.e at 2&3 mg/kg diazepam has more muscle relaxant effect than alprazolam and this result is statistically significant.* ***Conclusion:*** *This study was carried out to evaluate the muscle relaxant property of alprazolam which was compared with diazepam in mice by rotarod method test. It was concluded that the standard drug diazepam.has higher muscle relaxant effect than alprazolam.*

Keywords: *Muscle relaxant activity, diazepam, alprazolam, Rotarod method*

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I. Introduction

Muscle relaxant is actually a drug which affects skeletal muscle function and reduces the muscle tone. It may be used to alleviate symptoms such as muscle spasms, pain, and hyperreflexia. It mainly exerts it's pharmacologic effect centrally at the level of the spinal cord, the brainstem, or the cerebrum. The term muscle relaxant can be used to refer to two major therapeutic groups: neuromuscular blockers and spasmolytics [1, 2, 3]. Neuromuscular blockers act by interfering with transmission at the neuromuscular end plate and have no central nervous system (CNS) activity [4]. They are often used during surgical procedures as well as in intensive care and emergency medicine to cause paralysis. Spasmolytics, also known as centrally acting muscle relaxants, are used to alleviate musculoskeletal pain and spasms and to reduce spasticity in a variety of neurological conditions [5]. By 1943, neuromuscular blocking drugs became established as muscle relaxants in the practice of anesthesia and surgery. Muscle relaxants are used to treat acute muscle spasms, spasticity from upper motor neuron syndrome, torticolis, orthopedic manipulations and low back or neck pain, fibromyalgia, tension headaches and myofascial pain syndrome [6]. There are 2 main categories of skeletal muscle relaxants: antispastic (such as baclofen or dantrolene) for conditions such as cerebral palsy and multiple sclerosis and antispasmodic agents for musculoskeletal conditions [7]. Evidence is extremely limited to support the use of antispastic agents for musculoskeletal conditions, for which an antispasmodic agent is typically more appropriate. Although muscle relaxant are actually classified into one group, the Food and Drug Administration

*(*FDA*)* has approved just a few medications in this class to treat spasticity; the rest are approved to treat musculoskeletal conditions. There are benzodiazepines (BZD) which are sedative –hypnotics and also have muscle relaxant activity. While it can be used in patients with muscle spasm of almost any origin, it produces sedation in most individuals at the doses required to reduce muscle tone. The main effect of benzodiazepines is hypnosis, decrease anxiety, amnesia, centrally mediated muscle relaxation and anticonvulsant activity [8]. BZD act through specific BZD receptor which is an integral part of $GABA_A$ receptor Chloride channel complex. It enhances binding of GABA to $GABA_A$ receptor. They appear to increase the frequency of chloride channel opening mediated by GABA and cause hyperpolarisation and reduction in membrane excitability. Benzodiazepines like Diazepam are preferred in panic states and anxiety associated with organic disease. It possesses anxiolytic, anticonvulsant, hypnotic, sedative, skeletal muscle relaxant, and amnestic properties. Alprazolam is short-acting anxiolytic of the benzodiazepine class of psychoactive drugs. Clinical studies have shown that alprazolam has a side effect profile similar to that of other benzodiazepines, although drowsiness and light headedness occur less frequently than with diazepam. The present study was designed to assess the muscle relaxant activity of alprazolam and compare the muscle relaxant activity of alprazolam with diazepam using albino male mouse as experimental model.

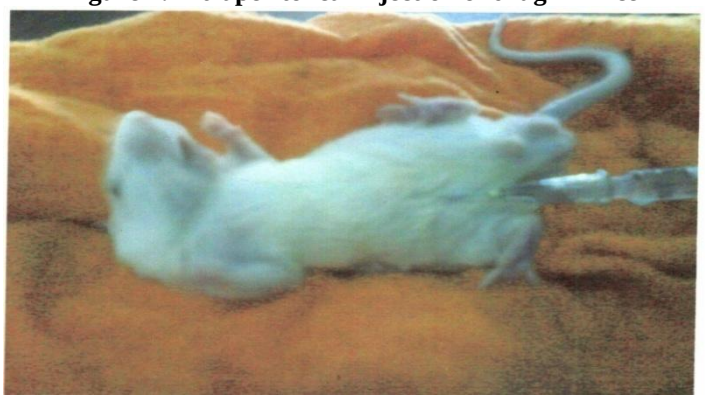
II. Materials And Methods

In the present investigation, free fall off method was used to identify the central muscle relaxant effect (Muscle grip strength) of drug in male albino mice. The skeletal muscle relaxation together with taming or calming effect of Benzodiazepines reduce anxiety and tension. The loss of muscle grip is an indication of muscle relaxation. This effect can be easily studied in animals using rotarod. There has been recently some renewed thinking about the importance of Rotarod method (Kinnard and Watzman 1996) [4]. Instead of just noting fall off time, more detailed observations should be made. It is important to observe the animal while it is walking on the Rotarod to see how many times the animal takes free ride. A free ride is defined as a revolution of the rod during which the animal holds on the rod, rather walks on it. There free ride could affect the calculations on drug responses. The difference in the fall off time from the rotating rod between the control and drug treated animal is taken as an index of muscle relaxation. The angle of the slope of the inclined plane or the rate of rotation of the rod should be adjusted such that a normal mouse can stay on the plane or on the rod for an appreciable period (3-5 min) of time.

Rotarod method: The white albino mice were selected, weighed and numbered. A total of 24 animals weighing about 30 grams were selected for the present study. They were divided into four groups each group consists of 6 mice. Animals which stay on the rotarod in between 2-5 minutes were included and others were excluded. An appropriate speed (15 rpm) on the rotarod is ideal and is used in the study. The rotarod is divided into several compartments; and the animal was placed one by one on the rotarod more than one mouse at a time were placed. Initially free fall off reading was taken before administration of drugs. The free fall off time was noted when the mouse falls from the rotating rod. A normal untreated mouse generally falls off with in 3-5 minutes. The reaction time was recorded after 30 minutes interval following administration of diazepam (2mg/kg) and Alprazolam (2mg/kg) administered separately through intraperitoneal mode of inoculation (Figure-1). Then reaction time was also recorded for 3mg/kg and 4mg/kg of Diazepam and alprazolam.

Statiscal analysis: Statistical analysis done in MS OFFICE Excel (2007) by applying one way ANOVA (Data analysis) and calculated the significance of drug. P value <0.05 indicates significant.

Figure 1: Intraperitoneal injection of drug in Mice



***Statistical methods*:** The significance of difference between treatment outcomes was analyzed by One-Way ANOVA. Statistical analysis was done with the help of statistical unit of the department of social and preventive

medicine. All statistical tests are two tailed and p value rounded to two decimal places. $p < 0.05$ was considered statistically significant.

III. Results

The present study was conducted in male albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of Diazepam, alprazolam. For initial screening of a drug, mouse is one of the best animals as it is easy to handle and can be used repeatedly since the animal is not sacrificed by rotarod method. Second choice of animal is rat. Table-1 was the control group where the animals were treated only with 0.2ml normal saline. In the present study it was found that the percentage of fall of free ride time for Diazepam was 8.30% with 2mg/kg (Table-2), 9.07% with 3mg/kg (Table-5) and 9.88% with 4mg/kg (Table-8). It was 8.29% with 2mg/kg (Table-3), 8.87% with 3mg/kg (Table-6) and 10.03% with 4mg/kg of alprazolam (Table-9) when assessed by rotarod method. At 2mg/kg dose the percentage of fall of free ride time for diazepam was 8.30% and for alprazolam is 8.29% which was almost same at this concentration (Table-4). At 3mg/kg dose the percentage of fall of free ride time for Diazepam is 9.07% whereas for alprazolam it was 8.87% (Table-7). The difference is slight but was more for Diazepam when compared with alprazolam. Diazepam has more muscle relaxant property. By applying one way ANOVA result came for this was significant. Diazepam was more potent and it reduces the muscle strength property which was more than alprazolam. At 4mg/kg the percentage of fall of free time for diazepam was 9.88% whereas for alprazolam it was 9.96% (Table-10).The difference was little bit more for alprazolam when it was compared with diazepam. By applying one way ANOVA result came for this was Insignificant. This study shows at this concentration alprazolam 4mg/kg body weight in mouse was slightly potent or somewhat similar to Diazepam at the same concentration and it reduces the muscle strength property (Figure-2).

Table 1: Control (C) group- Treatment with 0.2 ml of Normal saline

Fall of free ride time				
SNo.	**Body weight (mice)**	**Before Normal saline (sec)**	**After Normal saline(sec)**	**Mean value**
1	30gms	282	280	278.15
2	30gms	260	260	
3	30gms	283	282	
4	30gms	290	290	
5	30gms	276	275	
6	30gms	280	280	

Table 2: Detection of muscle relaxant activity of Diazepam at the dose of 2mg/kg body weight (S_1) using Rotarod method

			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Diazepam	280	240
2	30gms	Diazepam	260	225
3	30gms	Diazepam	280	235
4	30gms	Diazepam	280	240
5	30gms	Diazepam	275	230
6	30gms	Diazepam	278	238
Mean value: 255.05	**Percentage of fall of free ride time: 8.30%**	**Standard deviation: 7.84**	**Standard error: 3.20**	**P <0.05 Significant**

Table 3: Detection of muscle relaxant activity of Alprazolam at the dose of 2mg/kg body weight (T_{1a}) using Rotarod method

			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Alprazolam	290	220
2	30gms	Alprazolam	282	225
3	30gms	Alprazolam	279	235
4	30gms	Alprazolam	280	230
5	30gms	Alprazolam	277	238
6	30gms	Alprazolam	285	220
Mean value: 255.1	**Percentage of fall of free ride time: 8.29%**	**Standard deviation: 4.71**	**Standard error: 1.92**	**P <0.05 Significant**

Table 4: Comparison of percentage of fall of free ride time at effective dosages (2mg/kg) of Alprazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall	Mean	Standard	Standard error	P-value

		of free ride time		deviation		
Diazepam	2mg/kg	8.30%	255.05	7.84	3.20	P <0.05 Significant
Alprazolam	2mg/kg	8.29%	255.1	4.71	1.92	

Table 5: Detection of muscle relaxant activity of Diazepam at the dose of 3mg/kg body weight (S_2) using Rotarod method

			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Diazepam	278	235
2	30gms	Diazepam	260	220
3	30gms	Diazepam	280	230
4	30gms	Diazepam	278	234
5	30gms	Diazepam	275	228
6	30gms	Diazepam	280	237
Mean value: 252.92	**Percentage of fall of free ride time: 9.07%**	**Standard deviation: 7.65**	**Standard error: 3.12**	**P <0.05 Significant**

Table 6: Detection of muscle relaxant activity of Alprazolam at the dose of 3mg/kg body weight (T_{1b}) using Rotarod method

			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Alprazolam	282	218
2	30gms	Alprazolam	279	220
3	30gms	Alprazolam	290	230
4	30gms	Alprazolam	280	228
5	30gms	Alprazolam	285	235
6	30gms	Alprazolam	277	218
Mean value: 253.5	**Percentage of fall of free ride time: 8.87%**	**Standard deviation: 4.7**	**Standard error: 1.9**	**P <0.05 Significant**

Table 7: Comparison of percentage of fall of free ride time at effective dosages (3mg/kg) of Alprazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall of free ride time	Mean	Standard deviation	Standard error	P-value
Diazepam	3mg/kg	9.07%	252.92	7.65	3.12	P <0.05 Significant
Alprazolam	3mg/kg	8.87%	253.5	4.7	1.9	

Table 8: Detection of muscle relaxant activity of Diazepam at the dose of 4mg/kg body weight (S_3) using Rotarod method

			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Diazepam	280	230
2	30gms	Diazepam	260	215
3	30gms	Diazepam	280	220
4	30gms	Diazepam	275	225
5	30gms	Diazepam	280	230
6	30gms	Diazepam	278	235
Mean value: 250.67	**Percentage of fall of free ride time: 9.88%**	**Standard deviation: 7.84**	**Standard error: 3.20**	**P <0.05 Significant**

Table 9: Detection of muscle relaxant activity of Alprazolam at the dose of 4mg/kg body weight (T_{1c}) using Rotarod method

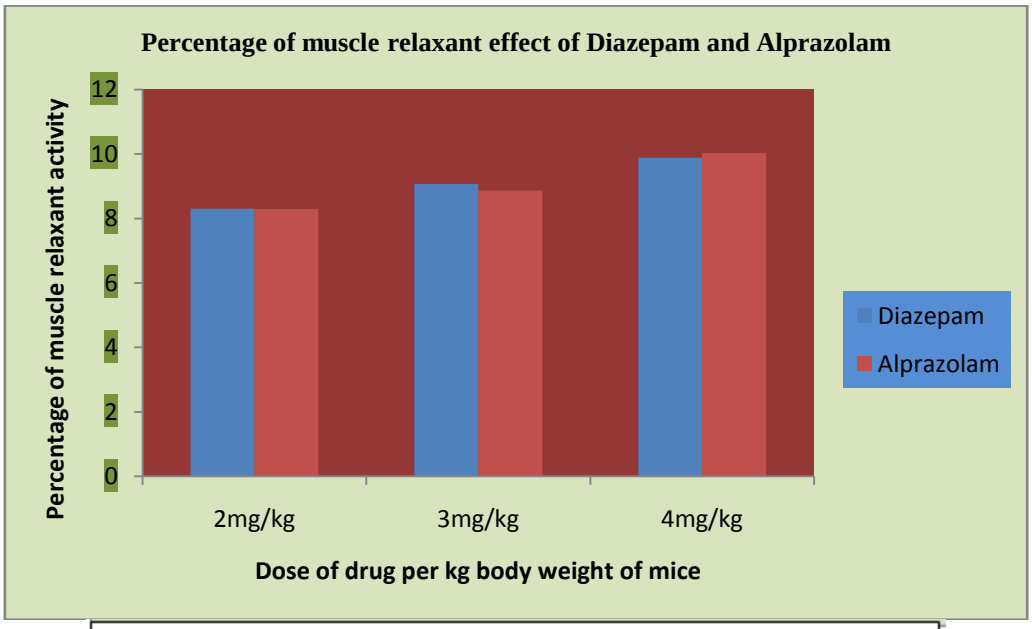
			Fall of free ride time	
SNo.	Body weight(mice)	Treatment	Before Normal saline(sec)	After Normal saline(sec)
1	30gms	Alprazolam	285	215
2	30gms	Alprazolam	280	218
3	30gms	Alprazolam	290	216
4	30gms	Alprazolam	278	225
5	30gms	Alprazolam	282	220
6	30gms	Alprazolam	280	214
Mean value:	**Percentage of fall**	**Standard**	**Standard error: 1.78**	**P <0.05**

250.25	**of free ride time: 9.96%**	**deviation: 4.37**		**Significant**

Table 10: Comparison of percentage of fall of free ride time at effective dosages (4mg/kg) of Alprazolam with Diazepam on Rotarod Method

Name of the drug	Dose	Percentage of fall of free ride time	Mean	Standard deviation	Standard error	P-value
Diazepam	4mg/kg	9.88%	250.67	7.84	3.20	P >0.05
Alprazolam	4mg/kg	9.96%	250.25	4.37	1.78	InSignificant

Figure 2: Percentage of muscle relaxant effect at the dose of 2mg/Kg, 3mg/Kg 4mg/Kg body weight in male albino mice



Formulae for % of fall of free ride time for 2mg/kg, 3mg/kg,4mg/kg of diazepam respectively.
C-S_1/C =100
C-S_2/C =100
C-S_3/C =100
Formulae for % of fall of free ride time for 2mg/kg, 3mg/kg,4mg/kg of alprazolam respectively.
C-T1a/C =100
C-T1b/C =100
C-T1c/C =100

IV. Discussion

Previous studies suggested that the CNS depression and non specific muscle relaxation effect can reduce the response of motor coordination. Increased muscle tone is common feature of anxiety states in human and may contribute to the aches and pains including headache often troublesome in anxious patients. The relaxant effect of benzodiazepines may therefore be clinically useful. A reduction of muscle tone appears to be possible without appreciable loss of coordination. Skeletal muscle relaxants are used to treat two different types of conditions like spasticity from upper motor neuron syndrome and muscular pains or spasms from peripheral musculo-skeletal conditions. In this study centrally acting skeletal muscle relaxants diazepam, alprazolam are used and muscle relaxant activity of alprazolam is compared with diazepam. The study was carried out in albino mice weighing 30 grams. Six mice were included in each group to study the muscle relaxant property in different concentrations such as 2, 3 and 4 mg/kg of diazepam, and alprazolam. At 2mg/kg dose the percentage of fall of free ride time for diazepam and alprazolam was almost same at the same concentration. At 3mg/kg dose the percentage of fall of free ride time for diazepam and alprazolam showed slight variations with diazepam having more muscle relaxant effect than alprazolam. The study also revealed that, alprazolam at 4mg/kg dose was slightly or some what similar potent than Diazepam at the same concentration and it reduces the muscle strength activity which is little bit more than Diazepam. This study was carried out to evaluate the muscle relaxant property of alprazolam which was compared with diazepam in mice by rotarod method test. It was found that the drug alprazolam has good muscle relaxant action similar to the drug diazepam. Veena et al. (2015) studied the centrally acting skeletal muscle relaxants diazepam and its muscle relaxant activity The study

was carried out in albino mice weighing 40gms. They found that diazepam demonstrated muscle relaxant property and considered to have maximum muscle relaxant property may be due to high lipid solubility [4]. In 1956, Dunham and Miya suggested that the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. This forced motor activity has subsequently been used by many investigators. The dose which impairs the ability of 50% of the mice to remain on the revolving rod is considered the endpoint. By this test the muscle relaxant potency in a series of compounds such as the benzodiazepines (Vogel *et al*) have been performed [9].

V. Conclusion

The present study was carried out to compare the muscle relaxant activity of alprazolam, and Diazepam given in different concentration in experimental model in rotarod test in albino mice. Diazepam and alprazolam were given in concentration of 2mg/kg, 3mg/kg and 4mg/kg body weight in rota rod method for each mouse in each group respectively. It was found that diazepam and alprazolam produced central muscle relaxant effect when assessed by rotarod test. On inter drug comparison of the two drugs it was found that, even though alprazolam has slightly higher muscle relaxant effect at increased concentration i.e at 4 mg/kg body weight as compared to drug diazepam, this result is statistically not significant. At decreased concentration i.e at 2&3 mg/kg diazepam has more muscle relaxant effect than alprazolam and this result is statistically significant. Hence it was concluded that the muscle relaxant activity of diazepam is higher than that of alprazolam. The authors declared "No conflict of interest"

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