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CNS Depressant and Muscle Relaxant effect of Ethanolic Leaf Extract of *Moringa Oleifera* on Albino Rats

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Abstract: *Moringa oleifera* is highly valued and ethnopharmacologically important plant. In today's world, 10-20% of adult population suffer from insomnia. The rampant use of skeletal muscle relaxants for both muscle spasm and spastic conditions make it almost impossible to escape from its side effects. *Moringa oleifera* is a widely distributed and easily cultivable plant. The leaves are enriched with nutrients and is a reservoir of phytochemical ingredients like flavonoids, terpinoids, saponins. Thus this plant can serve as an alternative to these problems.

The present study uses two experimental models. The CNS depressant action was studied in the actophotometer test and muscle relaxant by rotarod test. The albino rats were divided into six groups of 6 rats each. A total of 36 rats were used in each of the two experimental models. Group I: Control (normal saline given orally at 2ml/kg body weight); Group II: Standard (Diazepam 10 mg/kg p.o); Group III,IV,V,VI (EMO 50, 100, 200, 400 mg/kg respectively). Ethanolic extract of *Moringa oleifera* (EMO) leaves and diazepam were orally given 1 hour before the experiments.

Results were expressed as mean ± SE. ANOVA followed by post hoc tests were applied to both the experiments. In actophotometer, both standard (diazepam at 10 mg/kg p.o) and test drug (EMO 100,200,400 mg/kg) significantly reduced the locomotor activity. Similarly in rotarod the time of fall was also decreased by both standard and test drugs. Level of significance for both experiments were taken at $p<0.05$.

The data indicates that ethanolic leaf extract of Moringa oleifera has both CNS depressant and muscle relaxant activity
Keywords: CNS depressant, muscle relaxant, *Moringa oleifera*, leaves, extract, Muscle relaxant, locomotor activity , Ethanolic extract, *Moringa oleifera.*

Introduction:

It has been for more than 5000 years now that herbal extracts are being used for the treatment of diseases in the Indian subcontinent [1]. The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine [2]. According to the WHO data 80% of the population in developing countries rely on herbal medicine. Various authors have already described medicinal properties of Azadirachta indica seed oil [3]. Henceforth considering the gravity of the situation in developing countries the authors have decided to highlight

their research on the muscle relaxant and CNS depressant actions of another ethnopharmacologically important plant named *Moringa Oleifera*. *Moringa oleifera* belongs to the family of Moringaceae and is one among the 14 species [4]. The plant is native to India, Africa, Arabia, South Asia, South America, pacific and Caribbean Islands [5]. In some parts of the world it is known as 'Drumstick tree' or the 'Horse radish tree'. In Philippines it is known as 'Mothers best friend' as it increases the mother's milk production [6]. *Moringa oleifera* is easy to cultivate, multipurpose plant. It usually grows in hot dry land in tropical insular climate, being little affected by drought [7].The leaves are tripinnate and a rich source of amino acids, protein, vitamin A and C, calcium, potassium and natural antioxidants[8][9]. The phytochemical ingredients of the leaf revealed to be the storehouse of flavonoids, saponins, tannins, phenolic acids[4]. Previous literature studies have shown that these compounds may be responsible for CNS depressant and muscle relaxant activity[10].The leaves of *Moringa oleifera* (EMO) alsohasantiinflammatory[11],anticataleptic[12],antioxidant[13],antimicrobial[14],antihypertensive[15],hypocholesterolemic[16], antifungal[17], radioprotective[18] and antinociceptive[19] actions.

Skeletal muscle relaxants are agents that treat both muscle spasm and spasticity, acting as antispasmodic and antispasticity agents respectively. However this broad distinction is often overlooked[20].The modern day antispasmodic agents like cyclobenzaparin are used to treat to treat musculoskeletal conditions. Antispasticity agents like dantrolene are used to relieve muscle hypertonicity. The side effects of antispasmodic agents and antispasticity agents cause them to be used with caution [21]. Previous reports have shown that 10-20% of adults suffer from insomnia [22]. Hypnotic drugs are those psychoactive agents used to induce sleep. Sedative drugs are used to calm the patient. However the sedative and hypnotics are again subjected to notorious adverse effects like dependence and abuse liability [21]. Thus looking for an effective alternative has always been a priority in this regard.

We have already reported the analgesic effect of ethanolic leaf extract of *Moringa oleifera* [23]. The present study was conducted to evaluate the locomotor activity and muscle relaxant activity of this plant.

Material & Methods:

Materials

Collection of plant material

The leaves were collected for the local areas of Syampur, Bhubaneswar, Odisha, 751003 and its identity was confirmed by taxonomist Dr.P.C.Panda of Regional Plant Research Centre (RPRC), Bhubaneswar.

Preparation of extract

Fresh leaves were collected dried in shade and powdered. The powder was extracted with 90% ethanol using continuous hot air percolation method in a Soxhlet apparatus for 18 hrs. Extract filtered using Whitman filter paper no 1 and concentrated in rotary evaporator to yield a semi solid mass of 42 g (yield 8.4 % w/w). Extract stored in refrigerator at 4^0 C and used for oral administration[24].

Chemicals

Diazepam (Calmpose, Ranbaxy Laboratories Ltd, Solan, Nihalgarh ,India) ,and other solvent chemicals used were of analytical grade.

Animals

Wistar Albino rats of either sex (100-200 g) were randomly selected from the central animal facility. The animals kept at ambient temperature of 22 ± 1°C, 12hr light and dark cycle allowed. Food, water given *ad libitum.* Animals were acclimatized to laboratory conditions for 7 days prior to taking them for experimentation. The study was approved by the Institutional Animal Ethical Committee (IAEC) of Siksha O Anusandhan University, Bhubaneswar under the approval number 22/12/IAEC/SPS/SOA. All experiments and animal care were according to the CPCSEA and Good Laboratory Practice Guidelines. No animals were sacrificed at the end of the study.

Method :

It is a randomized control study. The animals were randomly divided into 6 groups with 6 rats each; Group I: Control (normal saline given orally at 2ml/kg body weight); Group II: Standard (Diazepam 10 ml/kg

p.o); Group III,IV,V,VI (EMO 50, 100, 200, 400 mg/kg respectively). The total number of rats used in each experiment was 36, so a total of 72 rats were used in this study.

Actophotometer : It consists of six built in light source and photo sensor and a digital counter to indicate locomotor activity. The device operates on photoelectric cells which are connected to the circuit with the counter. When the beam of light falling on the photocells is cut off by the animal, a count is recorded. Animals were placed in the actophotometer and their basal activity was recorded over the period of 10min. The test and the standard drug was administered 1 hr before the procedure and the recording taken after 1 hr. Decreased activity score was taken as index of CNS depression[25].

Rotarod is a horizontal metal rod coated with rubber, 3cm in diameter, put at a rotation of 25 rpm. The metal rod is about 50 cm above the surface to prevent the animal from jumping off the roller. The albino rats were placed on the revolving rod for a period of 10 min. Only those animals which remained in the revolving rod for atleast 1 min were taken in the study. The initial basal reading of the number of rotations covered by each animals before falling from the rotarod during this period was recorded. The test and standard compound was administered 1hr before placing the rats on the rotarod. The number of animals falling from the rotarod during this period was counted.

The percent animals falling from the rotarod within the test period was calculated for every test drug concentrations, standard and compared[13],[25].

Statistical Analysis :

Statistical analysis was done using one way ANOVA followed by post hoc Dunnett's T3 test in actophotometer test and ANOVA followed by post hoc Bonferroni's test in rotarod test using SPSS version 16. The results were tabulated as Mean ± SE.

Results:

In the Actophotometer, activity score for 10 min before drug administration and that after 1 hr of drug administration were compared among the six treatment groups. The cluster box plot (Figure 1) of activity score before and after drug administration reveal that except normal saline and EMO at 50 mg/kg there was a visible impact in the reduction of activity score after one hour of drug administration. There was no outliers or extreme values in the data, and the data confirms to the test of normality (Shapiro Wilk test) for all treatment groups. Therefore ANOVA was conducted. Descriptive statistics and the ANOVA results are furnished in Table 1.Mean activity score with 95% confidence interval is presented in figure 2.

Figure 1: Activity Score Before & After Drug Administration

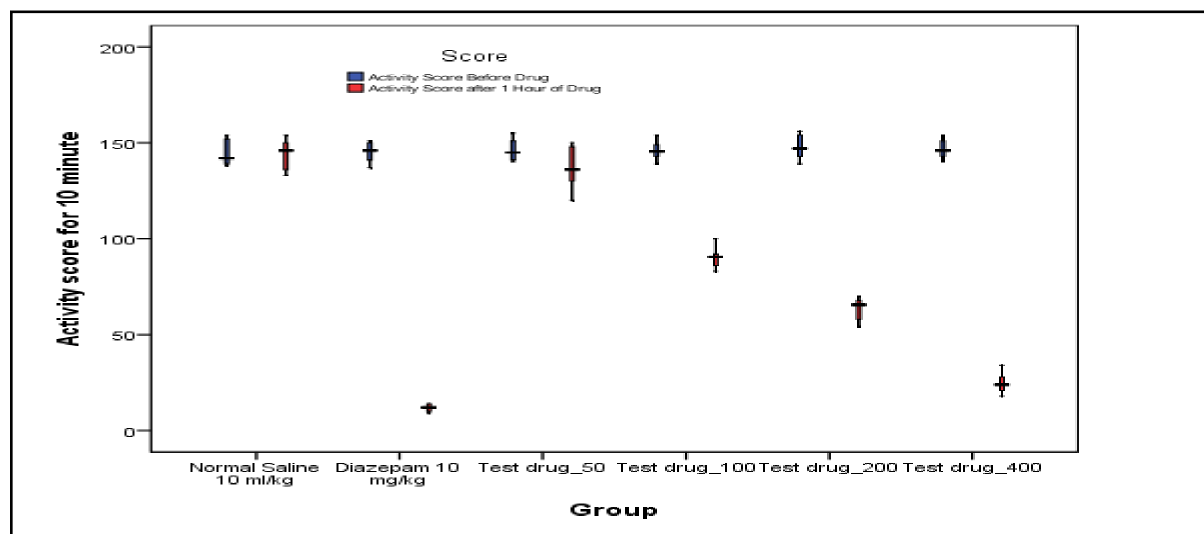


Table1: Descriptive and ANOVA for Activity Score Before and After Drug Administration

Variable	Group	Mean ± S.E	ANOVA	
			F Value	Sig. (P Value)
Activity score for 10 min before drug administration	Normal Saline 10 ml/kg	144.500 ± 2.825	0.211	0.955
	Diazepam 10 mg/kg	145.167 ± 2.242		
	Test drug_50	146.167 ± 2.386		
	Test drug_100	146.000 ± 2.098		
	Test drug_200	147.667 ± 2.741		
	Test drug_400	146.667 ± 2.108		
	Total	146.028 ± 0.93		
Activity score for 10 minutes after 1 hr of drug administration	Normal Saline 10 ml/kg	144.167 ± 3.351	355.343	0.000
	Diazepam 10 mg/kg	11.833 ± 0.872		
	Test drug_50	136.667 ± 4.695		
	Test drug_100	90.333 ± 2.376		
	Test drug_200	63.500 ± 2.604		
	Test drug_400	24.833 ± 2.344		
	Total	78.556 ± 8.631		

Figure 2: Comparison Activity Score Before & After Drug Administration (Error Bar)

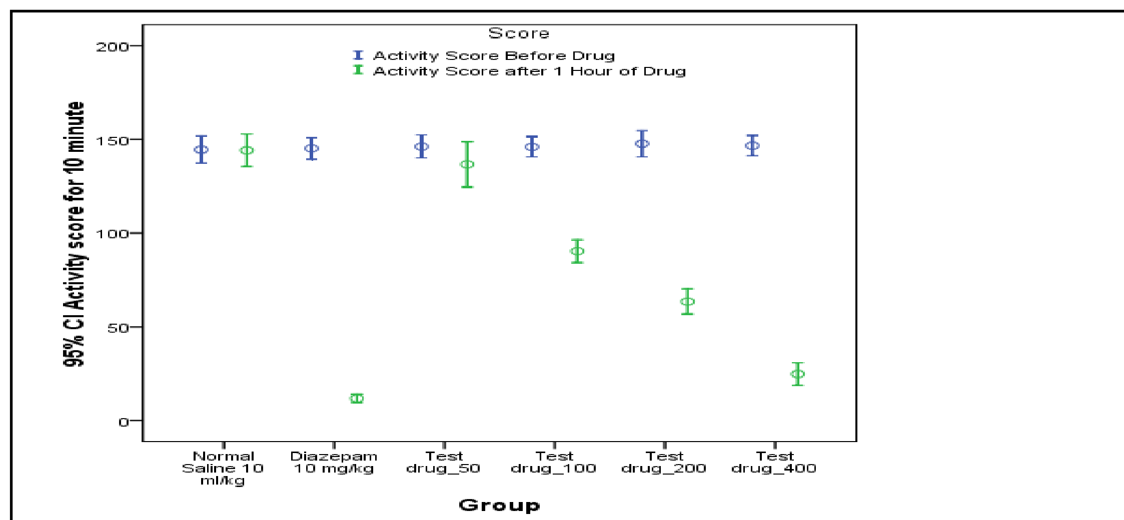


Table 1 and Figure 1 read together revealed the reduction of activity score after 1 hr of drug administration in comparison to the activity score before drug administration, except the normal saline and test drug at 50 mg/kg. In the Diazepam group before drug administration the mean level was 145.17±2.24 and after drug administration it reduced to 11.83±0.87. Test drug at 100mg/kg, the mean activity scores before and after drug administration were 146.00± 2.10 and 90.00±2.38 respectively. Similarly for 200mg/kg and 400 mg/kg, the reductions the reductions were 147.67±2.74 to 63.50±2.60 and from 146.67±2.12 to 24.83±2.34 respectively. Before drug administration there was no significant (p=0.955) difference in the mean level of activity score among the treatment groups. But 1hr after the drug administration there was a significant (p=0.000) difference among the mean activity score of treatment groups. Table 2 shows the post hoc (Dunnett's T3) test for pair wise multiple comparison of activity score after 1 hr of drug administration. The mean activity score of normal saline group was significantly (p=0.000) different in comparison to the Diazepam, test drug 100 to test drug 400 mg/kg. The activity score of diazepam 10 mg/kg was significantly (p=0.000) lower than all the test groups. The mean percent reduction in activity score before and after drug administration using ANOVA is presented in Table 3. The percentage reduction was highest for diazepam, 91.84% followed by the test drug 400mg/kg 83.04%. In the test drug 50 mg/kg insignificant % reduction or low percentage reduction of 6.57 was noted. The mean percentage reduction among different groups were statistically significant (p=0.000).

Table 2: Post Hoc (Dunnett T3) test for pairwise multiple Comparison of Activity Score after 1 hour of Drug dministration

(I) Group	(J) Group	Mean Difference (I-J)± S.E.	Sig.
Normal Saline 10 ml/kg	Diazepam 10 mg/kg	132.333 ± 3.462**	0.000
	Test drug_50	7.5 ± 5.768	0.925
	Test drug_100	53.833 ± 4.108**	0.000
	Test drug_200	80.667 ± 4.244**	0.000
	Test drug_400	119.333 ± 4.089**	0.000
Diazepam 10 mg/kg	Test drug_50	(-)124.833 ± 4.776**	0.000
	Test drug_100	(-)78.500 ± 2.531**	0.000
	Test drug_200	(-)51.667 ± 2.747**	0.000
	Test drug_400	(-)13.000 ± 2.501*	0.018
Test drug_50	Test drug_100	46.333 ± 5.262**	0.000
	Test drug_200	73.167 ± 5.369**	0.000
	Test drug_400	111.833 ± 5.248**	0.000
Test drug_100	Test drug_200	26.833 ± 3.525**	0.000
	Test drug_400	65.500 ± 3.337**	0.000
Test drug_200	Test drug_400	38.667 ± 3.504**	0.000

*p<0.05, **p<0.01

Table 3 Percent Reduction in Activity Score before and after drug Administration using ANOVA

Percent Reduction in Activity Score	Mean Reduction (%) ± S. E	ANOVA	
		F Value	Sig (P Value)
Normal Saline 10 ml/kg	0.223 ± 1.413	478.343	0.000
Diazepam 10 mg/kg	91.840 ± 0.62		
Test drug_50 mg/kg	6.568 ± 2.375		
Test drug_100	38.076 ± 1.774		
Test drug_200	56.886 ± 2.082		
Test drug_400	83.045 ± 1.68		
Total	46.106 ± 5.935		

Table 4: Multiple Comparisons of Percent Reduction in Activity Score after drug administration : Post Hoc Dunnette T3 test

(I) Group	(J) Group	Mean Difference (I-J) ± S.E.	Sig.(P Value)
Normal Saline 10 ml/kg	Diazepam 10 mg/kg	(-)91.61654 ± 1.54322**	0.000
	Test drug_50	(-)6.34517 ± 2.76382	0.403
	Test drug_100	(-)37.85259 ± 2.26788**	0.000
	Test drug_200	(-)56.66321 ± 2.51634**	0.000
	Test drug_400	(-)82.82174 ± 2.19556**	0.000
Diazepam 10 mg/kg	Test drug_50	85.27137 ± 2.45467**	0.000
	Test drug_100	53.76395 ± 1.87882**	0.000

	Test drug_200	34.95333 ± 2.17224**	0.000
	Test drug_400	8.7948 ± 1.79086*	0.023
Test drug_50	Test drug_100	(-)31.50742 ± 2.96431**	0.000
	Test drug_200	(-)50.31804 ± 3.15845**	0.000
	Test drug_400	(-)76.47657 ± 2.90936**	0.000
Test drug_100	Test drug_200	(-)18.81062 ± 2.73503**	0.001
	Test drug_400	(-)44.96915 ± 2.44314**	0.000
Test drug_200	Test drug_400	(-)26.15853 ± 2.67537**	0.000

*p<0.05, **p<0.01

Exploratory data analysis at the time of fall in rota-rod experiment revealed that the observation confirms to normality (Shapiro Wilk test). There was no extreme values and outliers in the data. Analysis of variance was conducted followed by post hoc (Bonferroni's test) for the comparison of mean time of fall among different treatment groups. Mean time of fall before drug administration was more or less of same level for different treatment groups (table 5) with p value 0.654.But there was significant (p=0.000) difference in the mean time of fall among the different treatment group after drug administration. After drug administration the time of fall in diazepam group registered a phenomenal fall from 90.17±3.19 to 19.5±1.09.The second highest reduction was observed in the test drug 400 mg/kg from 186.83±2.26 to 65±2.70. The fall in score was progressive with the doses of the test drug (Table 5). Figure 3 illustrates mean time of fall for different treatment groups. For diazepam the mean time of fall was very low which distinctively is different from others.In case of test drug there is a progressive decline of the time of fall with the increased dose of the test drug. Post hoc test for multiple comparison is presented in table 6.The time of fall in respect of diazepam is significantly lower from the test drug at all levels.

Table 5: Comparison of Mean Time of fall among Treatment Groups

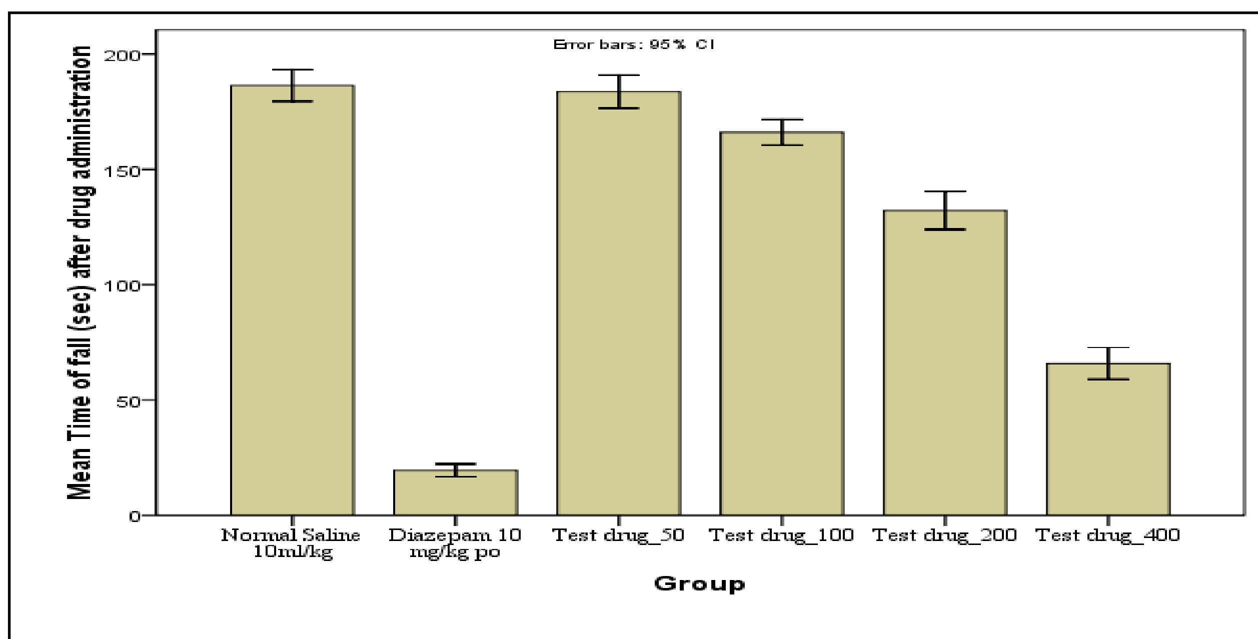
Variable	Group	N	Mean ± S.E	ANOVA	
				F Value	Sig. (P Value)
Time of fall (sec) before drug administration	Normal Saline 10ml/kg	6	185.500 ± 2.487	0.663	0.654
	Diazepam 10 mg/kg p.o	6	190.170 ± 3.188		
	Test drug_50	6	184.670 ± 2.275		
	Test drug_100	6	188.500 ± 1.821		
	Test drug_200	6	186.330 ± 2.654		
	Test drug_400	6	186.830 ± 2.257		
	Total	36	187.000 ± 0.989		
Time of fall (sec) after drug administration	Normal Saline 10ml/kg	6	186.330 ± 2.667	735.231	0.000
	Diazepam 10 mg/kg po	6	19.500 ± 1.088		
	Test drug_50	6	183.670 ± 2.789		
	Test drug_100	6	166.000 ± 2.160		
	Test drug_200	6	132.170 ± 3.229		
	Test drug_400	6	65.830 ± 2.701		
	Total	36	125.580 ± 10.636		

Table 6: Time of Fall Before Drug Administration

(I) Group	(J) Group	Mean Difference (I-J) ± S.E.	Sig.
Normal Saline 10ml/kg	Diazepam 10 mg/kg po	166.833 ± 3.581**	0.000
	Test drug_50	2.667 ± 3.581	1.000
	Test drug_100	20.333 ± 3.581**	0.000
	Test drug_200	54.167 ± 3.581**	0.000
	Test drug_400	120.5 ± 3.581**	0.000
Diazepam 10 mg/kg po	Test drug_50	(-)164.167 ± 3.581**	0.000
	Test drug_100	(-)146.5 ± 3.581**	0.000
	Test drug_200	(-)112.667 ± 3.581**	0.000
	Test drug_400	(-)46.333 ± 3.581**	0.000
Test drug_50	Test drug_100	17.667 ± 3.581**	0.000
	Test drug_200	51.5 ± 3.581**	0.000
	Test drug_400	117.833 ± 3.581**	0.000
Test drug_100	Test drug_200	33.833 ± 3.581**	0.000
	Test drug_400	100.167 ± 3.581**	0.000
Test drug_200	Test drug_400	66.333 ± 3.581**	0.000

*p<0.05, **p<0.01

Figure 3: Mean time of Fall Before & After Drug Administration (ErrorBar)



Discussion :

The modern day antispasmodic and antispaticity agents are subject to a lot of contraindications and side effects. The predominant side effects of antispasmodic agents are CNS depression and weakness, while of antispasmodic agents are sedation, inability to operate machinery and G.I disturbances. Apart from these they should be used with caution in elderly, children and in heart diseases[21]. Patients of insomnia rely on Benzodiazepines and newer Non Benzodiazepines to get relief. These drugs are notorious for their daytime fatigue, cognitive impairment and physical dependence and abuse liability[21].

Actophotometer is used for screening the locomotor and anti-anxiety activity in rodents, while the rota-rod for muscle relaxant activity. Locomotor activity indicates alertness and the decrease indicates sedative action[25]. The $GABA_A$ receptor complex is involved in sedation, muscle relaxant and anxiety in CNS. Various neurological and psychological disorders such as epilepsy, depression, Parkinson syndrome, Alzheimer's disease are involved with this receptor. Benzodiazepines like diazepam in the actophotometer test act by potentiation of the $GABA_A$ receptors, causing membrane hyperpolarization , ultimately leading to decrease in the firing rate of neurons in the brain or by directly acting on the GABA receptor where increased GABA neurotransmission has a damping effect on the stimulatory pathways causing a psychologically calming effect[26],[27]. Diazepam taken as a control here at dose of 10 mg/kg orally showed significant ($p<0.01$) CNS depressant and muscle relaxant activity. Ethanolic leaf extract of *Moringa oleifera* in this study could also act by the same mechanism that is as either GABA facilitatory or GABA mimetic action. Rota-rod test was first used in the screening of neurotoxicity by anticonvulsant drugs, but now it also predicts the motor inco-ordination caused by centrally acting drugs usually of the sedative and antipsychotic drug category[28]. EMO here showed significant ($p<0.05$) progressive decrease in the time of fall from the rotarod on increasing the dose.

Previous work has been done using the methanolic extract of *Moringa oleifera* leaf which revealed significant reduction in muscle relaxant activity by rotarod test[29]. The absolute alcoholic fraction of *Moringa oleifera* leaf juice showed significant reduction in activity score in actophotometer test[30]. The present study also corroborate to these findings.

Preliminary phytochemical screening revealed the presence polyphenolic compounds like, tannins, flavonoids, saponins, and alkaloids reducing sugars, triterpenoids in the plant extract[31]. The flavonoids present in the leaf extract can easily cross the blood brain barrier and exert various effects on the CNS, like memory, cognition and neurodegeneration[32],[33].Triterpinoid saponins, flavonoids have an agonistic action on $GABA_A$ receptor complex and hence may act like benzodiazepine like molecules[34],[35]. Thus these compounds may be responsible for its CNS depressant and muscle relaxant activity.

Conclusion:

The ethanolic extract of Moringa oleifera exhibited significant($p<0.05$) CNS depressant and muscle relaxant activity in a dose dependent manner. However further studies need to be conducted to know the exact mechanism of action of this plant, isolate the active ingredients responsible for this activity and extend the study the study to know the anticonvulsant, sedative, hypnotic, and general anaesthetic actions.

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