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Effect of Ethanolic Extract of Datura Stramonium on Muscle Relaxant Activity by Docking Study of Gallic Acid and Gabba a Receptor and Rotarod Method in Mice

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Abstract

Background: The current study examined Datura stramonium leaves' ability to relax muscles. The extract's ability to relax muscles was evaluated using a variety of animal models. The study's objective was to compare the skeletal muscle relaxant properties of diazepam with the aqueous extract of Datura stramonium leaves in albino mice.

Materials and Methods: Twenty Swiss albino mice, of either sex, weighing between 100 and 150 g, and aged 5 to 6 weeks were used. Two distinct dosages were chosen following acute toxicity tests. They separated the animals into four groups. Different dosages of the EEDS were administered to the first group, which was retained as the control (normal saline), the second as the standard (diazepam), and the remaining two as Test I and Test II.

Results: At a maximal effect of 20 mg/kg i.p., the EEDS demonstrated substantial action. The EEDS (100, 250, 500, and 1,000 mg/kg) and EEDS (200, and 400 mg/kg) were deemed safe in the acute toxicity screening test. Rotarod activity is conducted with skeletal muscle relaxant action (motor coordination). Analysis of variance and Dunnett's multiple comparison tests were used for statistical analysis.

*Conclusions***:** According to our research, EEDS has skeletal muscle relaxant properties.

Keywords: Rotarod, muscle relaxant, *Datura stramonium*, soxhlet apparatus

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Introduction

Muscle is one kind of soft tissue found in animals. Muscles are meant to provide force and motion. They are primarily responsible for posture maintenance and modification, the movement of the organism, and the movement of internal organs, such as the contraction of the heart and the peristalsis that transports food through the digestive system. Tendons, ligaments, and other fibrous layers make comprise the body's deep fascia.[1]

Comparing the limbs of a horse and a mole is one of the simplest ways to comprehend this. The insertion point is positioned to maximize force (for digging) in the latter and to maximize speed (for running) in the former.[2] Often, deeper muscles, including those involved in posture, are controlled by brain stem and basal ganglia nuclei.[3]

A. Neuro muscular blockers:

Most neuromuscular blockers function by blocking transmission at the end plate of the neuromuscular junction. When a nerve impulse typically reaches the motor nerve terminal and initiates an influx of calcium ions, acetylcholine-containing vesicles are exocytosed.[4] Acetylcholine then diffuses across the synaptic cleft. Muscle contraction results from the end plate depolarizing, which permits K+ ions to leave the cell and Na+ and Ca2+ ions to enter. Following depolarization, the cholinergic molecules are removed from the end plate region and hydrolyzed enzymatically by acetylcholinesterase.[5] Instead, depolarize the receptor until it loses its sensitivity and can no longer initiate an action potential that would otherwise cause a muscle contraction.[6] The natural ligand acetylcholine, which often consists of two acetylcholine molecules joined end-to-end by a rigid carbon ring structure, as in the nondepolarizing drug pancuronium, shares structural similarities with both of these classes of neuromuscular blocking drugs.[7]

Muscle relaxant for treatment of neuromusculoskeletal conditions:

Common musculoskeletal disorders that result in discomfort and muscle spasms include myofascial pain syndrome, fibromyalgia, stress, headaches, and mechanical neck or low back pain. if a muscle spasm occurs under specific conditions. It has to do with local factors influencing the affected muscle groups.[8]

B. Spasmolytics

The brain impulses that cause muscular contractions depend on the ratio of synaptic excitation to inhibition that a motor neuron receives. Inhibition is enhanced by mimicking or intensifying the actions of naturally occurring inhibitory substances, such GABA.

Because they can function at the level of the cortex, brain stem, spinal cord, or all three, they have historically been referred to as "centrally acting" muscle relaxants. However, it is now known that not all of the compounds in this class, such as dantrolene, have CNS activity, therefore this nomenclature is inaccurate.[9]

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Every medication has adverse effects, such as drowsiness and lightheadedness, and no medication has been shown to be better than another. In general, muscle relaxants have not been approved by the FDA for long-term use. Patients feel more refreshed when they wake up when this period of sleep is extended. Better sleep may also help those who suffer from fibromyalgia.[10]

Mechanism:

As a result of the central nervous system's increased inhibition, diazepam and other benzodiazepines interact with the GABAA receptor. Most individuals get drowsy at the dosages required to reduce muscle tone, although it can be used on patients who have muscle spasms from almost any reason.[11] Baclofen may also lessen pain in patients by blocking the release of substance P in the spinal cord.[12] [8]

Side effects:

Strong drugs known as muscle relaxants have a number of negative effects, including the potential for paralysis and heart failure.[10] Respiratory failure may occur when baclofen is administered intrathecally. Spasticity in the upper motor neuron syndrome may be caused by a variety of brain or spinal cord illnesses.[13] An anti-inflammatory drug could be useful when there is a lot of tissue damage and edema. The findings of several studies have suggested a combination of an analgesic and a muscle relaxant. is more effective than similar doses of an analgesic alone in treating acute musculoskeletal problems.[14]

Methadology

Plant collection and authentification

The whole Datura stramonium plant was collected from Dundigal in the Medchal Malkajgiri region of Telangana, India, in March 2024. The plant's validity was confirmed by the botanist. Making an extract The coarsely powdered, shade-dried leaves of the Datura stramonium plant were macerated with 70% v/v ethanol for seven days. The solvent was filtered away after the extraction procedure. Evaporation After the dried extract has been filtered onto china plates and let to evaporate, it is used for further examination.

Identification of phytochemical constituents

Ethanolic leaf extracts of *Datura stramonium* was subjected to qualitative tests for the identification of various active constituents. Leaves of *Datura stramonium* were collected and extracted with 70 % v/v ethanol by continuous maceration process. The nature, organoleptic characters were presented in table1.

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Table 1: Extractive yield of plant extracts

Plant name	Parts used	Solvent used	Method of extraction	Colour and nature of the extract
Datura stramonium	Leaves	Ethanol	Maceration	Dark Green

Pharmacological Studies

Animals

Albino mice of 25 to 30 g were obtained from Hyderabad. They were housed in standard cages at room temperature (25±2^{o}C) and provided with food and water ad libitium.

Drugs and Chemicals

- *Datura stramonium* leaf extract.
- Standard drug: Diazepam(10mg).

Acute Toxicity Study and Dose Selection for Animal Screening:

To evaluate acute toxicity, three female mice that were not pregnant and were between the ages of 8 and 12 weeks were used. The animals were not allowed to eat for the whole night prior to the experiment, but they were allowed unlimited access to water. The OECD 423 principles were followed throughout the procedure. Ethanol and aqueous extracts were administered orally in four different dosages: 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg. Each animal was checked independently for at least half an hour following the first treatment, and then intermittently over the next 24 hours, with special attention during the first 4 hours and daily for a total of 14 days [15]. tremors, convulsions, limb tone, twisting, abdominal tone, pinna reflex, corneal reflex, irritation, and terror, diarrhea, Urination, Heart rate, Respiratory, Pupil size and Skin color.[16]

Rotarod method:

A horizontal, 3 cm-diameter, rubber-coated metal or wooden rod is attached to a motor that is configured to turn at a rate of two revolutions per minute to form the device [22]. Because the 75 cm long rod is divided into three sections by plastic discs, four mice can be examined simultaneously. The rod is placed around 50 cm above the table top to prevent the animals from leaping off the roller. The cages underneath the sections aid in restricting the animals' movement when they fall off the roller. Mice that weigh between 25 and 35 grams are used for the device's pretest.[17, 19] The baseline reading, or the total number of falls in three minutes, was taken after each mouse was placed independently on the rotarod. The mice are placed on the spinning rod after the test substance has been administered orally.[18]

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Mice were divided into four groups consisting of three animals each.

- Group I: Treated with 1 % Acacia (1 ml/kg, orally) and was kept as control
- Group II : Treated with Diazepam (10 mg/kg, intraperitoneally) and was kept as standard.
- Group III: Treated with the ethanolic *Datura stramonium* leaf extract (200 mg/kg, orally).
- Group IV: Treated with the ethanolic *Datura stramonium* leaf extract (400 mg/kg, orally).

Figure 1: Test sample administration

Animals who spent two minutes or more in low consecutive trials on a rotarod (25 rpm) were selected for testing. Repeat the test for two hours after the test drug or control vehicle is provided, 30 minutes later. By comparing the mice in the treatment and control groups' fall off times from the rotating rod, muscle relaxation was assessed.[20, 21]

Docking study of Gallic acid and GABBA $_A$ Receptor

Investigating the interaction between gallic acid and the GABA A receptor as a protein and ligand was the aim of this study. To analyze the interaction, MGL tools, Autodock Vina, Biovia Discovery Studio, and Open Bable were downloaded from their respective websites. A search of the literature revealed that Datura stramonium contains gallic acid [(23)]. It has been demonstrated that skeletal muscle relaxants may target the GABA A receptor. After obtaining the structure of gallic acid from Pubchem (Pubchem CID 370) in SDF format, Open Bable was utilized to convert it to pdb format[(25)]. The structure of the GABA A receptor was obtained from the Protein Data Bank, with PDB ID 6X3W(24). The protein was processed and visualized in Biovia Discovery Studio by adding polar hydrogens, removing extraneous ligands and amino acid chains, and saving it in PDB format. It was saved in PDBQT format and assigned an AD4-type atom.

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Results

Preliminary phytochemical screening

In this study ethanol extract *Datura stramonium* showed positive to following phytochemical constituent's total glycosides, alkaloids, flavonoids. Presence of Glycosides, Tannins, Flavonoids, Fixed oils and fats, Alkaloids, Total Phenolics Carbohydrates, Gums and mucilase, Protein and amino acids, Saponins

Acute Toxicity Study and Dose Selection:

Acute toxicity in non-pregnant female mice aged 8–12 weeks was evaluated using OECD guideline 423. Mice are watched for many signs throughout the observation period, such as attentiveness, irritability, and responsiveness to touch and pain. Both ethanolic and aqueous leaf extracts of Datura stramonium were shown to be non-toxic up to 2000 mg/kg and to cause no deaths. No more than 5000 mg/kg. No behavioral or mortality changes were seen in female mice.[22]

Muscle relaxant activity:

To investigate the muscle relaxant property in the mice, EEDS extract was given to them. Mice were given two distinct dosages, and the falloff time was noted thereafter.

Rotarod method:

The mice were divided into four groups, each group consists of three animals. The usual drug, diazepam 10 mg/kg, significantly ($p<0.05$) reduced the fall-off period in mice with muscle relaxant effect. At a dose of 200 mg/kg, *Datura stramonium* leaf extract decreased the fall off time in comparison to the control; however, a significant reduction was noted at a dose of 400 mg/kg.

Table 2: Effect of *EEDS* on muscle relaxant activity using rotarod method in mice

Treatment	Dose	Fall of time(seconds)				
		0 min (b.f)	30min	60min	90min	120min
Group I (1 % acacia)	1ml/kg	474.5±0.32	480±0.02	476±0.98	428±0.68	478±1.62
Group II (Diazepam)	10mg/kg	403.5±0.02	7.58±0.03	19.6±0.67	32.8±0.7	42±0.89
Group III (EEDS)	200mg/kg	482±0.02	103±0.03	115±0.87	120±1.15	132±0.68
Group IV (EEDS)	400mg/kg	362±0.06	86±0.02	94±0.32	103±1.01	116±0.74

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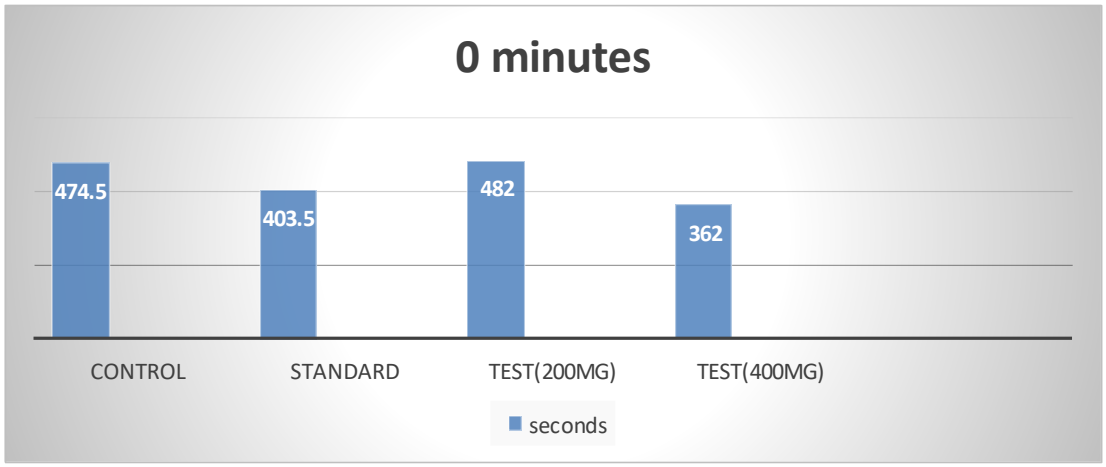

Figure 2: Effect of *Datura stramonium* on muscle relaxant activity at 0 minutes

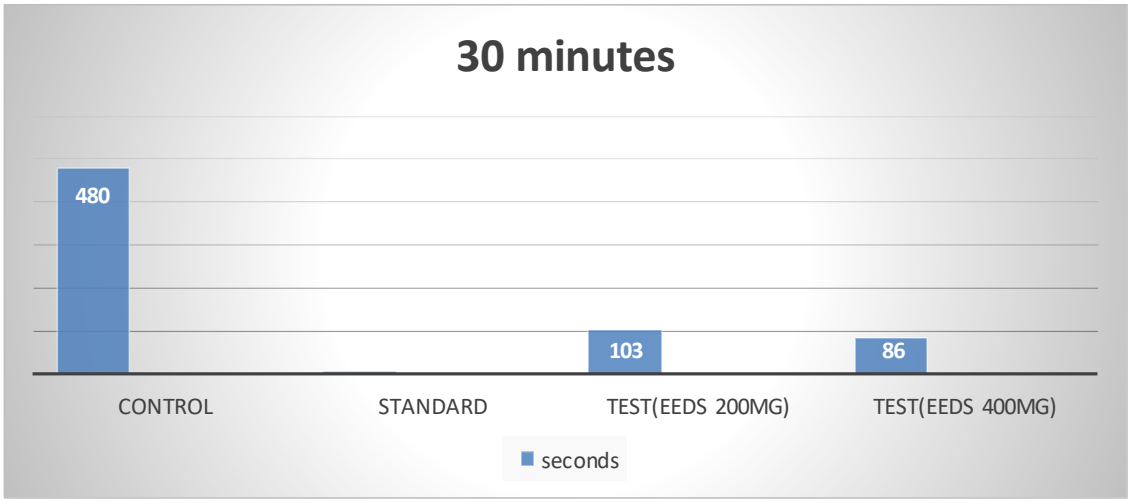

Figure 3: Effect of *Datura stramonium* on muscle relaxant activity at 30 minutes

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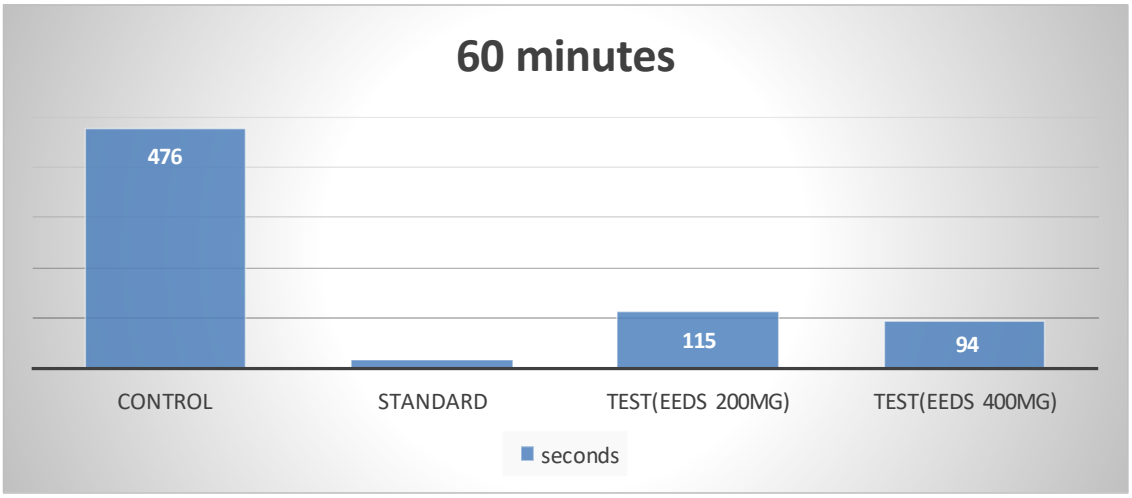

Figure 4: Effect of *Datura stramonium* on muscle relaxant activity at 60 minutes

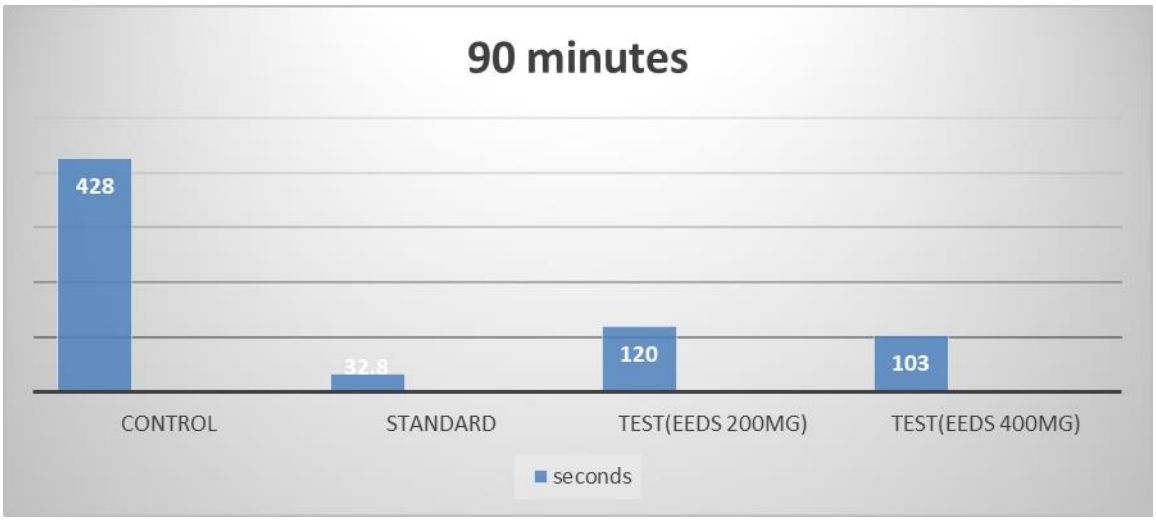

Figure 5: Effect of *Datura stramonium* on muscle relaxant activity at 90 minutes

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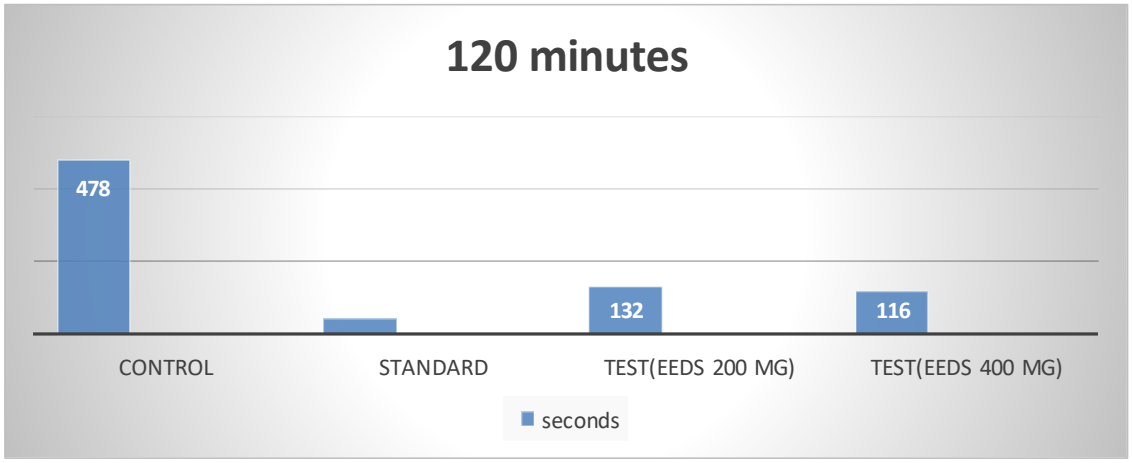

Figure 6: Effect of Datura stramonium on muscle relaxant activity at 120 minutes

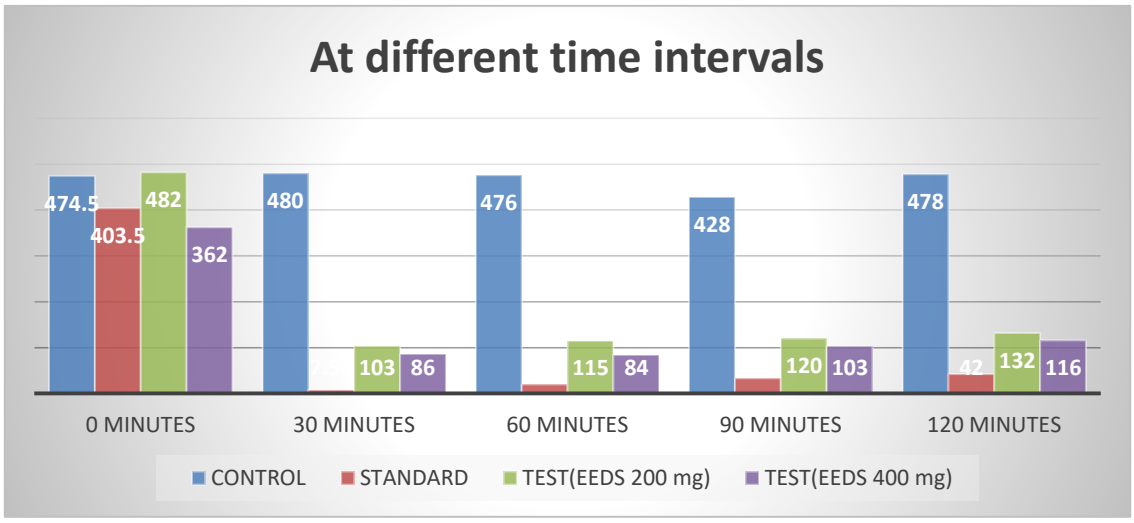

Figure 7: Effect of Datura stramonium on muscle relaxant activity at different timings in different doses

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Docking study of Gallic acid and GABBA $_A$ Receptor

Table 3: Docking studies were performed in 9 modes, and the results are given in the table below:

Mode	Affinity (kcal/mol)	Distance from rmsd l.b.	Best mode rmsd u.b.
1	**-5.8**	**0.000**	**0.000**
2	**-5.8**	**0.002**	**2.402**
3	**-5.6**	**2.011**	**2.811**
4	**-5.6**	**2.068**	**3.355**
5	**-5.6**	**1.254**	**4.158**
6	**-5.5**	**2.007**	**3.215**
7	**-5.5**	**1.995**	**3.467**
8	**-5.5**	**1.985**	**3.653**
9	**-5.5**	**1.984**	**3.794**

rmsd – root mean square deviation, l.b. – lower bound u.b. – upper bound

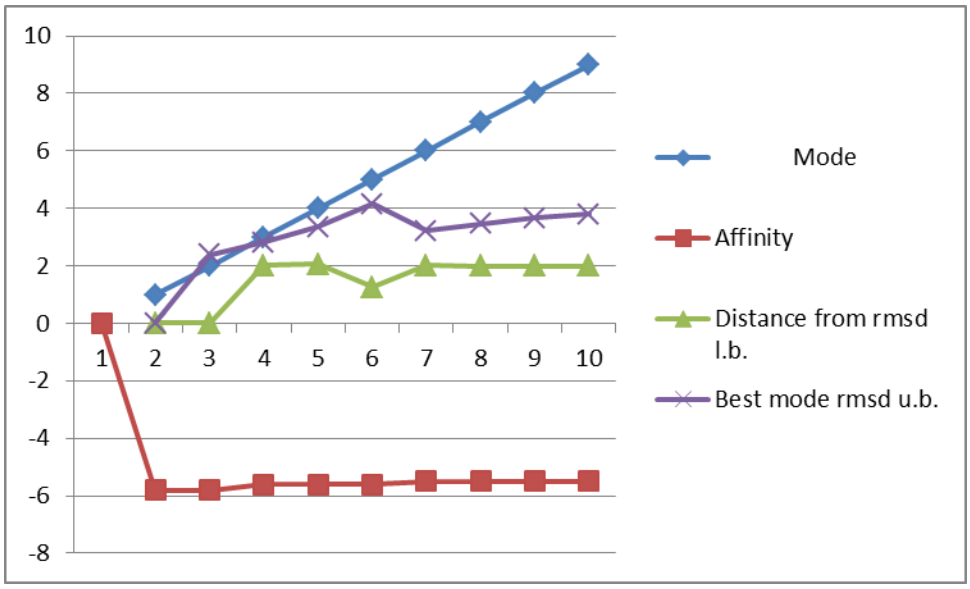

Figure 8: Effect of Datura stramonium on Docking studies were performed in 9 modes

Statistical analysis:

All values are expressed as Mean±SEM (n=6). $p < 0.05$ when compare to control. Statistical comparison was performed by using One-way ANOVA.

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Discussion

The purpose of this study was to assess the ethanolic extract of Datura stramonium's muscle relaxant properties. It is not exaggeration to say that plant extracts have potent medicinal qualities and may successfully treat a variety of diseases. In recent years, there has been an increase in interest worldwide in the use of herbal plants as medicines or health supplements. In order to investigate the muscle relaxant activity in mice, Datura stramonium was selected as the plant. The study was carried out on Albino mice using a single muscle relaxant model.

In the rotarod model, the falloff time was noted for every animal group. The falloff times for the control animals were 474, 476, 428, and 478 seconds at 0 minutes, 480 seconds, 60 minutes, 90 minutes, and 120 minutes, respectively. 403 seconds at 0 minutes, 7 seconds at 30 minutes, 19 seconds at 60 minutes, 32 seconds at 90 minutes, and 42 seconds at 120 minutes were the falloff timings for the usual group. The fall off time decreased for every animal in the standard group. The falloff times were 482 seconds at 0 minutes, 482 seconds at 30 minutes, 103 seconds at 60 minutes, 115 seconds at 90 minutes, and 132 seconds at 120 minutes for the test group that was given 200 mg/kg of EEDS.

There is a little decrease in the falloff time when compared to the control and standard groups. The falloff times were 362 seconds at 0 minutes, 86 seconds at 30 minutes, 94 seconds at 60 minutes, 103 seconds at 90 minutes, and 116 seconds at 120 minutes for the test group that was given 400 mg/kg of EEDS. The muscle relaxant effect of EEDS (400 mg/kg) was significant ($p<0.01$) in comparison to the standard. A decrease in falloff time was seen in the first hour and then in the second, as this effect was almost the same as the norm.

Conclusion

The results showed that mice's falloff time was reduced when they were given an ethanolic extract of Datura stramonium. Different EEDS doses were administered. The initial 200 mg/kg dosage resulted in a little decrease in falloff time, whereas the second 400 mg/kg dose caused a considerable decrease. The falloff duration was significantly decreased by the common drug, diazepam. The rotarod model demonstrated a significant reduction in fall off time when the standard was given, but this effect was not evident at the initial dosage (200 mg/kg) of the ethanolic extract of Datura stramonium. EEDS (200 mg/kg) caused a little decrease in falloff time in mice compared to the control group; this effect differs greatly from the norm.

It was also discovered that the falloff time decreased when the second dosage of the plant extract was compared to the standard and control groups. A considerable decrease in falloff time was noted when EEDS (400 mg/kg) was contrasted with EEDS (200 mg/kg). The impact of a muscle relaxant was seen for two hours. The falloff time was noticeably shorter during the first 30 and 60 minutes, and measurements taken after 90 and 120 minutes showed that the muscle relaxant effect was waning. This effect first causes a decrease in muscle relaxant activity, followed by a little increase in muscle relaxant action an hour later, much like the test in standard groups.

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At a dosage of 200 mg/kg, a very little muscle relaxant effect was seen; this effect increased when the amount of Datura stramonium ethanolic extract was increased.

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