Skeletal Muscle Relaxant Potential of *Annona reticulata* L. leaf extract in Swiss albino mice- a pre-clinical study

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ABSTRACT

Aim of the experiment: The present study was aimed at the investigation of skeletal muscle relaxant activity of *Annona reticulata* L. leaf ethanolic extract by using in vivo screening models.

Method: The skeletal muscle relaxation pontial of the *Annona reticulata* L. leaf extract was investigated using models such as Rota-rod, chimney test, actophotometer, and grip strength. The activity by three dose levels of the extract (low, medium, and high) was compared with the activity produced by standard (diazepam) and control groups of mice.

Results: The activity of 400mg/kg (high dose) was found to be 54.92±10.35 in the actophotometer experiment, the fall-off time in rota-rod and chimney test were found to be 92.7±3.1 and 15.50±0.763 respectively. The high dose level showed a significant loss of grip strength (147.32±3.39) when compared to control (205.4±5.3), medium (194.75±7.91), and low (182.35±7.06) dose levels, revealing muscle relaxation potential of the extract at higher dose levels.

Conclusion: Dose-dependent and significant skeletal muscle relaxant activity was observed in all the in-vivo models in animals treated with the plant ethanolic extract when compared to control and standard treatments.

1. INTRODUCTION

Skeletal muscle relaxants are a heterogeneous group of drugs which refer to two major therapeutic groups: neuromuscular blockers and spasmolytics [1,2]. Neuromuscular blockers mainly interfere with transmission at the neuromuscular plate and they have no central nervous system (CNS) activity.The action of spasmolytic agents involves enhancing the level of inhibition or reducing the level of muscle excitation. Diazepam, a benzodiazepine react with $GABA_{A,}$ promotes the binding of GABA which is the inhibitory neurotransmitter in brain, increase in chloride conductance across the neuronal cell membrane occurs, reducing the arousal of the cortical and limbic system and result in sedation property [3,4,5].

Annona reticulata Linn.belongs to family Annonaceae. Approximately 119 distinct Annona genus species (Annonaceae), have been described. It is widely grown in India and its fruit is edible. The old system of medicines, this plant was reported to have pharmacological activities such as antifungal, anticancer, spasmolytic, anticonvulsant, antimalarial, anthelmintic, and anti-syphilitic [6]. The present study was aimed to study the effectiveness of *Annona reticulata* L. leaf extract to produce skeletal muscle relaxation [7].

2. MATERIAL AND METHODS

2.1. Plant material

The leaves of *Annona reticulata L.* were collected during the month of April-May from Mangalore. The leaf was then authenticated by Dr. Jyothi Miranda, Associate Professor & HOD, Department of Botany, St. Aloysius College, Mangalore, Karnataka. The leaves were shade dried and coarse powdered for extraction.

2.2. Preparation of plant extract

The leaves were washed from adhering dust and other material and then dried under the shade for 30 days. After verifying the dryness,the leaves were pulverized in an electric grinder. The coarse powdered obtained was placed in an extraction chamber along with the ethanol as the extracting solvent for around 7 days at room temperature with occasional stirring. After 7 days the content was filtered by using a muslin cloth and

the marc was pressed. This process was repeated 3 times. All the fractions were combined and the ethanol was subjected to evaporation. The thick slurry obtainedwas evaporated until dry extract was obtained and stored in the desiccators for further usage.

2.3. Preliminary Qualitative phytochemical analysis

Preliminary phytochemicals test of the ethanolic extract of dried leaves of *Anonna reticulata* L. was performed for the presence of various active principles (steroids, flavanoids, glycosides, triterpenoids, and alkaloids) using standard procedures [8].

2.4. **Animals**

Albino mice of weighing 20-30g of either sex, 4-6 weeks, were obtained from NUCARE animal house, Nitte (deemed to be university) Deralakatte, Mangalore. The mice were appropriately grouped and then sheltered in distinct cages. They were allowed free access to a standard dry pellet diet and water *ad libitum.*The investigation was done in accordance to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and the researchwork was permitted and approved by the Institutional Animal Ethics Committee (IAEC).

2.5. **Experimental design:**

In the present study animals were divided into five groups consisting of six mice in each group.

Group I : Control (0.9 % saline).

Group II : Standard drug (Diazepam, 5mg/kg)i.p

Group III :Ehanolic extract of *Annona reticulata* L. (100mg/kg) p.o for 7 days.

Group IV :Ehanolic extract of *Annona reticulata* L. (200mg/kg) p.o for 7 days.

Group V :Ehanolic extract of *Annona reticulata* L. (400mg/kg) p.o for 7 days.

On the 8th day, after an hour of the test drug administration, the animals were taken for the following test for screening of skeletal muscle relaxant activity [9,10,11].

2.5.1. **Rota-rod**

Rota-rod was used to evaluate motor coordination produced by drugs in animals. The mice were trained before the experiment to remain for 300sec on a rod with diameter 2.5cm, rotating at 20 rpm. The animals were placed in the four paws on the rotating bar, which is 2.5 cm in diameter and 25 cm high from the floor. The animals were observed for a period of five minutes. The difference between the fall-off time of the mice before and after treatment was considered as the index of muscle relaxation[12,13].

2.5.2. Chimney Test

The "test de la cheminee" was introduced by Boissier*et al.,(*1960) as a simple test for muscle relaxant activity. Glass cylinders (30 cm in length) called chimney were used for this test. The cylinder was kept in horizontal position and mark was created near 20 cm at the end. The mice was kept in a forward direction. Then the mice were allowed to move till the other end. When it is reaches the other end the cylinder was kept in a vertical position and it was allowed to climb downwards. The time required by the mouse to climb backwards to the top of the cylinder was noted [14].

2.5.3. **Actophotometer**

Actophotometer was used to evaluate the locomotor activity of mice produced by drugs in animals. Most of the centrally acting drugs influence locomotor activity in animals. The locomotor activity can be easily measured using actophotometer which operates on photoelectric cells that were connected in the circuit with a counter. The animals were individually placed in the activity cage for 10 minutes. The basal activity score of all animals were recorded, and the difference in the activity before and after treatment of drug was considered as the index of locomotor action [15,16].

2.5.4. Grip strength

The test helps to assess muscular strength in rodents which can be influenced by muscle relaxant compounds. In a preliminary experiment, the animals were tested for their normal grip strength by exposing them to

horizontal thin metallic wire suspended about 30 cm into the air, which they immediately grasp with the forepaws. The mouse was released to hang on with its forelimb. Normal animals were able to catch the wire with the hind limbs and to climb up within 5 sec. only animals those which fulfill this criterion were included in the experiment. After the drug administration, the animals were checked for its ability to climb on the wire with the hind limbs with 5 minutes. The animals were monitored for every 15 minutes for 2 hours. The animals which were not able to climb or which fall off were considered to be impaired by the effect of the drug. Then the animals were observed for behavioral changes in the cages [17].

2.6. Statistical analysis:

The data is represented as Mean±SEM and was analyzed by using one way analysis of variance (ANOVA), then Dunnette's test carried out using the version 5 of the Graph Pad prism program. A statistically significant value of P is less than 0.05 was considered.

3. RESULTS:

3.1. Preliminary phytochemical analysis of ethanolic extract of *Annona reticulata L.*

In the preliminary phytochemical screening, the leaves of *Annona reticulata L.*showed and confirmed the presence of steroids, flavanoids, glycosides, triterpenoids, and alkaloids.

3.2. Rota-rod

In the present study, it was found that the ethanolic extract of the leaves of *Annona reticulata L.*on oral administration exihibit muscle relaxant activity at dose of 400mg/kg and the fall of time was found to be 92.7±3.1 seconds compared to standard drug Diazepam treated animals which showed 21.1±0.98 seconds of fall of time. The extract of *Annona reticulata* L.shows skeletal muscle relaxant activity in a dose-dependent manner, ($p<0.05$).

Table no :1 Effect of *Annona reticulata L.*on skeletal muscle in mice

Group	Treatment	Fall of time (Sec.)
I	Control	213.8±2.74
II	Standard (Diazepam)	21.1±0.98
III	Ethanolic extract 100mg/kg	141.5±2.83
IV	Ethanolicextract 200mg/kg	123.7±2.15
V	Ethanolic extract 400mg/kg	92.7±3.1

All the values are expressed as mean±SEM. n=06 animals in each group..a=p<0.05 when compared with Control b=p<0.05 when compared with Standard, using one way ANOVA using SPSS software.

3.3. Chimney test

In the present study, it was found that the ethanolic extract of the leaves of *Annona reticulata* on oral administration at a dose of (400mg/kg), showed a decrease in fall of time which is comparatively significant than the low and medium dose levels of the extract.

Table no :2 Effect of *Annona reticulata* L. on chimney test.

Group	Treatment	Fall of time (sec)
I	Control	34.833±1.13
II	standard	2±0.365
III	Ethanolic extract 100mg/kg	28.00±0.577
IV	Ethanolic extract 200mg/kg	26.33±0.881
V	Ethanolic extract 400mg/kg	15.50±0.763

All the values are expressed as mean±SEM. n=06 animals in each group.a<p,0.05 when compared with Control b=<p,0.05 when compared with Standard, using one way ANOVA using SPSS software.

3.4. Actophotometer

Group treated with diazepam (5mg/kg) served as the standard group showed significant skeletal muscle relaxant activity. At a dose of 100mg/kg, 200mg/kg, and 400mg/kg treated group of animals showed skeletal muscle relaxant activity in a dose-dependent manner.

Table no:3 Effect of *Annona reticulata* L. on actophotometer

Group	Treatment	Basal activity
I	Control	409±56.36
II	Standard	138.835±41.16
III	Ethanolic extract 100mg/kg	397.9±43.43
IV	Ethanolic extract 200mg/kg	377.835±78.63
V	Ethanolic extract 400mh/kg	54.9185±10.35

All the values are expressed as mean±SEM. n=06 animals in each group. a<p,0.05 when compared with control b<p,0.05 when compared with standard, using one way using SPSS software.

3.5. Grip Strength

The animals treated with extract of *Annona reticulate* L. in various dose levels showed lose of grip strength in a dose-dependent manner.

Table no :4 Effect of *Annona reticulate* L. on grip strength meter

Group	Treatment	Grip strength (gm)
I	Control	205.4±5.3
II	Standard	127.0±4.67
III	Ethanolic extract 100mh/kg	182.35±7.06
IV	Ethanolic extract 200mg/kg	194.75±7.91
V	Ethanolic extract 400mg/kg	147.32±3.39

All the values are expressed as mean±SEM. n=06 animals in each group..a<p,0.05 when compared with Control b<p,0.05 when compared with Standard, using one way ANOVA using SPSS software.

4. DISCUSSION:

The present study was conducted to screen the skeletal muscle relaxant activities of leaf extract of *Annona reticulata L*. The skeletal muscle relaxant activity was evaluated by the rota-rod model, chimney test model, actophotometer model and grip strength meter. The results showed a dose-dependent increase in muscle relaxation with different doses ofethanolic extract of *Annona reticulata* L. which was significant when compared to the control.The ethanolic extract of *Annona reticulata* L.showed muscle relaxation activity at doses of 100, 200, and 400 mg/kg. At a dose of 400 mg, the muscle relaxant activity was comparable to the standard drug diazepam. Animals treated with Diazepam at a dose of 5 mg/kg body weight showed a significant lack of motor coordination, sedation, and muscle relaxant activity. Diazepam is a centrally acting skeletal muscle relaxant that acts by enhancing the effects of GABA. GABA is the most potent inhibitory neurotransmitter in the CNS. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs mediate their action through GABA. Therefore, the ethanolic extract of *Annona reticulata* L. may act by potentiating GABAergic inhibition in the CNS through membrane hyperpolarization causing a decrease in the firing rate of critical neurons in the brain. The rota-rod is one of the most commonly used animal models of skeletal muscle relaxant activity and has been validated for use with both rats and mice. Rota-rod used to evaluate motor coordination produced by drugs in animals. The study revealed that relaxation of muscle in animals treated with ethanolic extract indicated by the reduction in fall of time. In the Chimney test model, animals were not able to hold on to the mesh in the inverted position upon treatment with plant extract and a decrease in the muscle contraction declined the holding time.Actophotometer was used to evaluate the locomotor activity in mice. Most of the centrally acting drugs influence the locomotor activity in animals. The animals treated with a high dose of extract showed significantly reduced the motor coordination. The effects of drugs, toxins, muscle relaxants, disease, ageing or neural damage on muscle strength may be assessed using grip strength meter. The grip strength meter allows the study of

neuromuscular functions in rodents by determining the maximum force displayed by an animal. The grip strength test was used to assess the muscular strength in rodents which can be influenced by muscle relaxant compounds. The extracts of *Annona reticulata* L.in various dose levels showed a gradual decrease in the grip strength in a dose-dependent manner.

5. CONCLUSION

This study was carried on to investigate the skeletal muscle relaxant activity of ethanolic extract of *Annona reticulata L leaves*. The skeletal muscle relaxant activity was evaluated using Rota-rod, Chimney test, Actophotometer and Grip strength meter. *Annona reticulata* L. extract exhibited skeletal muscle relaxant activity in a dose dependent manner. These observations provide pharmacological support on the use of the *Annona reticulata L.* as muscle relaxant. In the future, this work can be extended by using with wider dose range of the extract to confirm the activity of *Annona reticulata L* extract. Furthermore, the isolation and characterisation of the phytochemical constituents responsible for pharmacological activity can be done and molecular level mechanism of the drug activity can be carried by estimating the levels of various chemical mediators .

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7. CONFLICT OF INTEREST:

The authors declare no conflict of interest.

8. FUNDING / SOURCE

Nil.

9. AUTHOR CONTRIBUTION

First author carried out the experimental work, under the guidance of second author. Third author contributed valuble suggestions.

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