Evaluation of Skeletal Muscle Relaxant Activity of Musa Acuminata Pulp Extract in Wister Rats

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***Abstract*—Skeletal muscle relaxant activity of the methanolic fruit pulp extract of *Musa Acuminata* was investigated by testing the effect of the extract on Wistar rat. The evaluation of in-vivo skeletal muscle relaxant activity in rats was done using Rota rod model & Actophotometer model. Experiments were carried on female Wistar rats and the animals were randomly allotted to the different control and test groups. *Musa acuminata* has been broadly using as traditionally medicine in several countries. Taxonomically *Musa Acuminata* belong to family Musaceae. In the present study, pulp powder of *Musa Acuminata* were subjected to phytochemical evaluation.. The powdered plant material was extracted by Soxhlet extractor in methanol and acetone.**

Phytochemical testing of extracts discovered presence of glycosides, flavonoids, carbohydrates, tannins, phenolic, flavonoids etc. Anti- oxidant activity was carried out by 2, 2- diphenyl-1-picrylhyrazyl (DPPH) assay. DPPH scavenging assay were performed to evaluate the antioxidant activity which was found maximum at 50 microgram concentration for methanolic extracts.

The extract was administered orally at a dose of 250mg/kg and 500mg/kg. Diazepam in a dose of 10 mg/kg (i.p) was used as a standard. The results showed that methanolic extract of *Musa Acuminata* shows significant skeletal muscle relaxant activity as compared to standard drug at the doses of 250mg/kg & 500mg/kg.

***Keywords*—Skeletal Muscle Relaxant activity, Musa Acuminata, Acto photometer, Rota rod apparatus, Muscle relaxant activity.**

I. INTRODUCTION

Skeletal muscle relaxants are drugs that act peripherally at neuromuscular junction/muscle fiber itself or centrally in the cerebrospinal axis to cause paralysis or reduce muscle tone. The neuromuscular blocking agents are used primarily in conjunction with general anesthetics to provide muscle relaxation for surgery, while centrally acting muscle relaxants are used mainly for painful muscle spasms and spastic neurological conditions.

There are several types of muscle relaxers, and they work in different ways. It's important to talk to your healthcare provider about the risks and benefits of these medications. In general, these agents are associated with significant side effects. Aim of this study is to overcome the side effect of synthetic medicine [1].

The present study was conducted to evaluate the skeletal muscle relaxant activity of *Musa Acuminata* plant.

Musa Acuminata belongs to the *Musaceae* family distributed widely in Asia. India is regarded as the major center of origin for more than 300 types of banana cultivars out of the 600 types of bananas. *Musa Acuminata* has been traditionally used to treat various diseases and ailments such as fever, bronchitis, allergic reactions, sexually transmitted infections, and some non- communicable diseases. All parts of the plant including fruit, stem, pseudo stem, flower, leaf, sap, inner trunk, inner core, and root have found their use in traditional medicine. Phytochemical investigations reveal the presence of chemical constituents such as carbohydrates phenolic, vitamin C, flavonoids like (quercetin, proanthocyanidins, catechin, proteins, alkaloid, tannins, saponins, flavonoids, steroid. This work was an attempt to explore Skeletal Muscle Relaxant activity of an Methanolic extract of Musa Acuminata in Swiss albino rat. it was found that MEMA shows skeletal muscle relaxation[2].

II. MATERIALS AND METHODS

Plant Material –
The fresh ripe pulp of *Musa acuminata* was washed thoroughly using tap water and wiped using a clean cloth. The succu-lent parts of the banana pulp were cut into about two-centimeter thickness. Next, the

cut pulp was allowed to dry in the oven at 50°C for seven days. The dried pulp was powdered using electric Grinder and kept at 4°C in a tight-capped bottle.

Preparation of Extract-
Selection of solvent:
On the basis extractive value and nature of phytochemical present in drug and literature review solvents were selected for the extraction of the fruit pulp of Musa Acuminata like Acetone, Methanol, Distill Water.

Selection of extraction method:
Study of literature survey revealed that most of the chemical constituents of the plant extract are heat stable, most of the chemical constituents required for the activity are active in these solvents extracts and most of the researchers selected continuous hot extraction method for plant extraction, Soxhlet extractor is very essential with less time usage and high efficiency, solvent penetrates faster to the plant. It is most convenient method. on that basis that Soxhlet extraction method was selected for extraction of pulp powder of plant.

Preliminary phytochemicals screening-
Phytochemical screening of the Methanolic extract of Ripe fruit pulp of *Musa acuminata* was performed for the presence of various active principles (tannins, saponins, flavonoids, quinines, glycosides, proteins, saponins, triterpenoids, phenol, alkaloids) using standard procedures.

IAEC Approval:

Selection and procurement of animals:
The experiment was performed with the approval of Institutional Animal Ethics Committee (IAEC) following guidelines of CPCSEA. The Female Wistar rats with 180-200 gm body weight were selected for study using Rota rod model and Actiphotometer. The Animals were allowed to acclimatize for a period of 7days, housed under standard conditions of temperature (25±2°C) and relative humidity (30%–70%) with a 12:12 light-dark cycle, fed with pellet diet and water[3].

Animals used:
Female Wistar rats – 24

Route of administration:
Standard: i.p
Test: P. O

Evaluation of skeletal muscle relaxant activity:
1) Rota rod model (motor coordination):
The Rota rod apparatus consists of a metal rod (3 cm diameter) coated with rubber attached to a motor with the speed adjusted to 20 rotations per minute. The rod is 75 cm in length and is divided into 5 sections by metallic discs, allowing the simultaneous testing of 5 rats. The rod is in a height of about 50 cm above the tabletop in order to discourage the animals from jumping off the roller.
Cages below the section serve to restrict the movements of the animals when they fall from the roller. Swiss albino rats underwent a pretest on the apparatus. Only those animals, which had demonstrated their ability to remain on the revolving rod (20 rpm) for 5 min, were used for the test. Swiss albino rats were divided into four groups consisting of six animals each.
Group I served as control which received saline solution, animals of group II received standard drug Diazepam at a dose of (10mg/kg, i.p.) while Group III & IV received the MEMA at a dose of 250 and 500 mg/kg, p.o.
30 mins after the injection of test material or standard drug the same test was repeated at interval of 30 min. animals were placed on the rotating rod and fall off time i.e., when the animal falls from the rotating rod, was recorded, which was taken as grip strength[4].

Table 1: Animal Grouping for Rota rod model.

Sr. No	Groups	Treatment	Dose	Route of administration
1	Control	Distill water	-	p. o
2	Standard	Diazepam	10mg/kg	i.p
3	Test1	MEMA	250mg/kg	p. o
4	Test2	MEMA	500mg/kg	p. o

Evaluation Parameters:
Fall of time from the rotating rod before treatment.
Fall of time from the rotating rod after treatment.
Percentage reduction in fall of time from the rotating rod.

2) Locomotor activity (Acto photometer):
The spontaneous locomotor activity was assessed with the help of an Acto photometer as described by Idris et .al, 2015 with minor modifications. Each animal was observed for a periodof 5 min in a square closed field area (30 cm × 30 cm × 30 cm) equipped with six photocells in the outer wall. Interruptions of photocell beams (locomotor activity) were recorded by meansof a six digits' counter.

Group I served as control which received saline solution, animals of group II received standard drug Diazepam at a dose of (10mg/kg, i.e.) while Group III & IV received the MEMA at a dose of 250 and 500 mg/kg, p.o[5].
To see the locomotor activity, the Acto photometer was turned on and each rat was placed individually in the activity cage for 5 min. The basal activity score for all the animals was noted. After 60 min of administration of control, standard, test extract, the activity score for 5 min was observed. The difference in the activity, before and after drug administration, was noted. The percentage decrease in motor activity was calculated[6].

Table 2: Animal Grouping for Actiphotometer Model:

Sr. No	Groups	Treatment	Dose	Route ofadministration
1	Control	Distill water	-	p.o
2	Standard	Diazepam	10mg/kg	i.p
3	Test1	MEMA	250mg/kg	p. o
4	Test2	MEMA	500mg/kg	p. o

Table 3: Preliminary phytochemical screening of methanolic extract of fruit pulp of *Musa acuminata*-

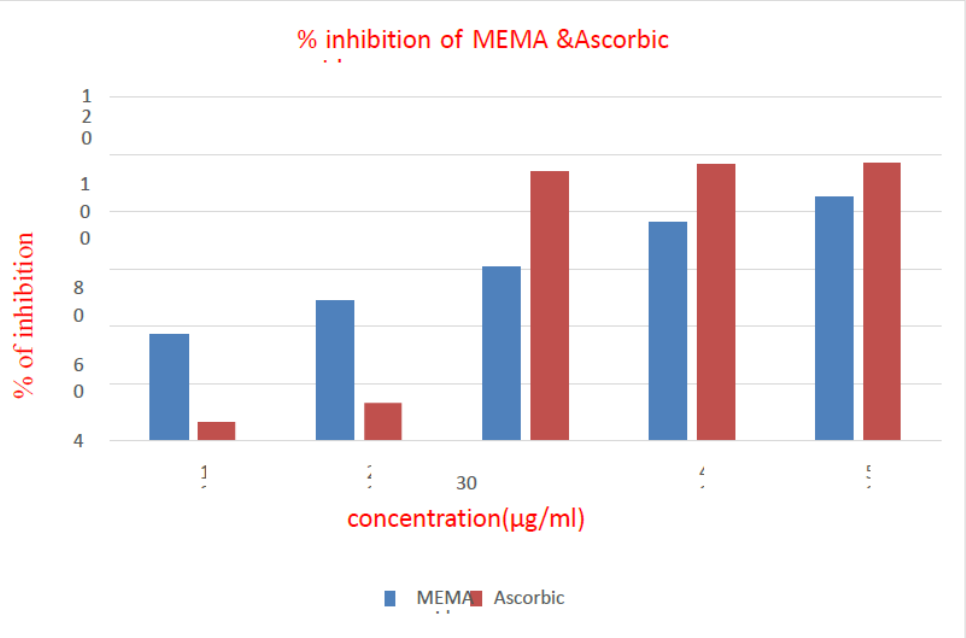
Test for carbohydrate		
Chemical test	Observation	Inference
1)Molish test	Violet ring at junction	+
2)Fehling's test	Red ppt	+
3)Benedicts test	Orange color	+
Test for protein		
1)Biuret test	No violet ring	–
2)Millions test	White ppt	+
3)Test for protein containing Sulphur	Blackish solution	+
Test for amino acids		
Ninhydrin test	Bluish color	+
Test for steroids		
1)Salkowski test	Golden yellow color	+
2)Libermann Burchard test	Red color	–
Test for saponins		
1)foam test	Foam formation	+

2)froth test	Formation of foam	+
Test for flavonoids		
1)Shinoda test	No color	+
2)lead aetate test	Yellow color	+
3)sulphuric acid test	Orange color	+
4)alkaline reagent test	Yellow color	–
5)leucoanthocyanidin test	Red ppt	+
Test for tannins and phenolic		
1)lead acetate test	White ppt	+
2)dilute test	Black color	–
3)ferric chloride test	Red color	–
4)bromine water test	Discoloration of bromine	+

Pharmacological screening of plant extracts -
In-vitro antioxidant activity –

Table No.4: DPPH Scavenging activity of plant extract & standard -

Sr.no	Conc. (µg/ml)	% inhibition	
		MEMA	Ascorbic acid
1	10	37.05	6.51
2	20	48.99	13.17
3	30	60.62	94.10
4	40	76.12	96.43
5	50	85.11	96.74



Graph No 1: % inhibition of plant extract &standard

In DPPH Scavenging assay method, the %inhibition of methanolic extract of *Musa acuminata* at 517nm has been recorded at different concentrations of 10, 20, 30,40, 50µg/ml respectively. The result was compared with Ascorbic acid as a reference standard and Methanolic extract of *Musa acuminata* showed very significant % inhibition close to reference standard. methanolic extract of *Musa acuminata* shows highest % inhibition activity 85.11% at 50µg/ml concentration

Table no:5 Effect of methanolic extract of *Musa acuminata* on muscle relaxant activityin rats.

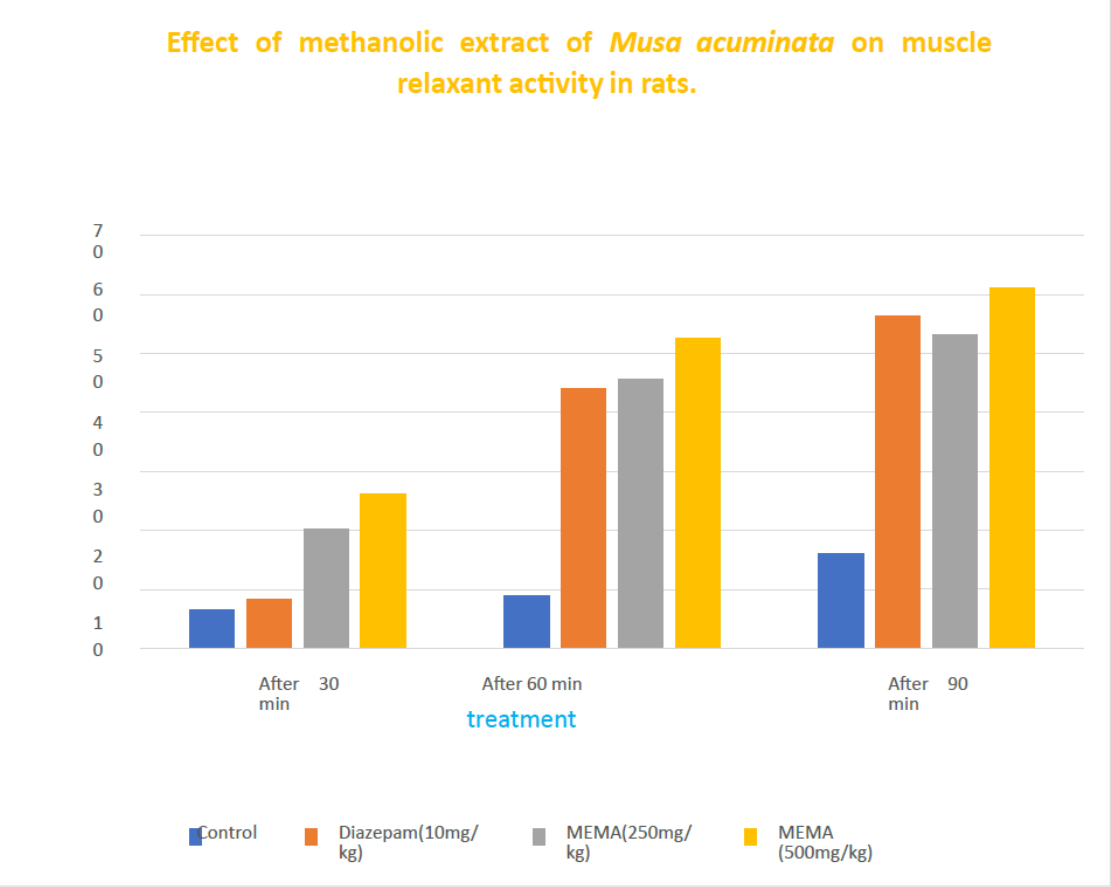
Treatment	Fall of time (mean ±SD)				% reduction in fall oftime		
	Before treatment	After 30min	After 60min	After 90min	After30 min	After60 min	After90 min
Control	27.6±2	25.8±1.72	25.16±1.23	23.16±1.86	6.521	8.84	16.04
Diazepam(10mg/kg)	26.03±1.62	24.26±1.92	14.76±12.6	11.53±3.9	8.38	44.02	56.27
MEMA (250mg/kg)	27.16±3	21.16±8.42	14.8±2.08	12.7±2.48	20.28	45.50	53.24
MEMA (500mg/kg)	27.16±2.6	20.03±8.47	12.88±2.02	10.56±2.04	26.25	52.57	61.11

% reduction in fall of time: Wa-Wb/WA ×100%

Where

Wa = mean fall off times before treatment

Wb = mean fall off times after treatment



Graph No 4: Effect of methanolic extract of *Musa acuminata* on muscle relaxant activityin rats

For muscle relaxation MEMA showed significant reduction in the time spent by the animals on the revolving rod when compared to the standard ($p<0.0005$). the results are summarized in table 15. The standard drug diazepam (10mg/kg) showed increase in muscle relaxation that is 8.38,44.02

&56.27 after 30,60,90 min of treatment. Two different doses of MEMA (250mg/kg &500mg/kg) showed a dose dependent increase in muscle relaxation that is 20.28 & 26.25 after 30 min of treatment. Two different doses of MEMA (250mg/kg &500mg/kg) showed a dose dependent increase in muscle relaxation that is 45.50 & 52.57 after 60 min of treatment. Two different doses of MEMA (250mg/kg &500mg/kg) showed a dose dependent increase in muscle relaxation that is 53.24 & 61.11 after 90 min of treatment. maximum muscle relaxation was observed with 500mg/kg of MEMA. The result from the Rota rod test showed that the MEMA reduced the motor coordination of the tested animal.

Table No: 6 Effect of methanolic extract of *Musa acuminata* on locomotor activity of rod

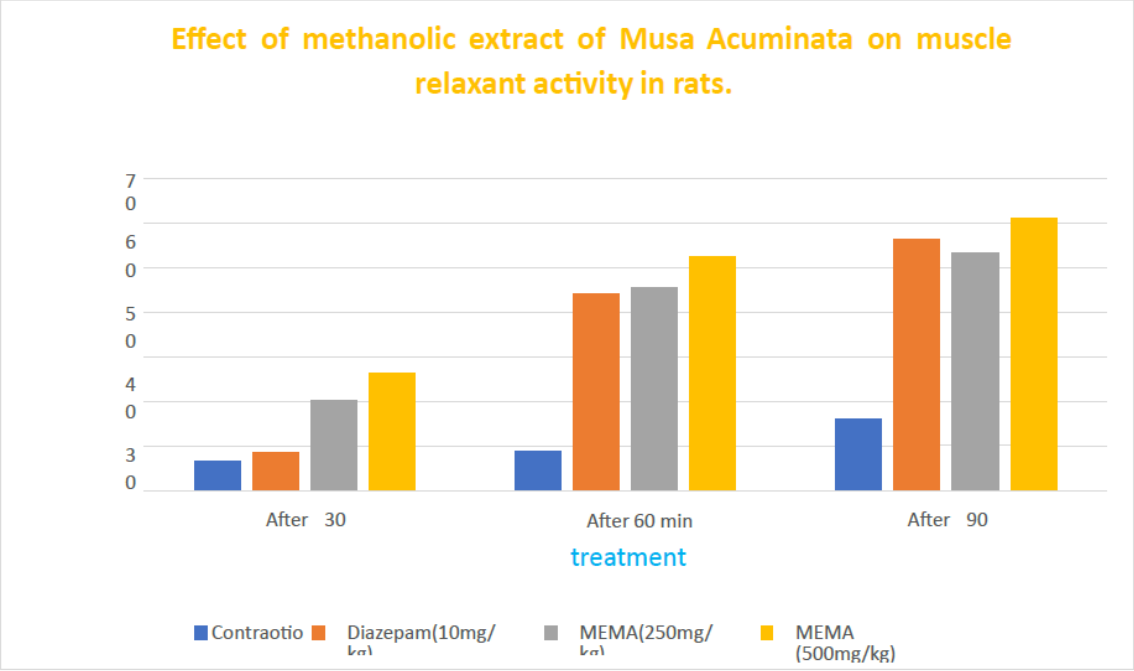
Treatment	(Mean ± SD) Actiphotometer scores		% reduction in locomotor activity
	Before treatment (0 min)	After treatment (60 min)	After 60 min
Control	22.66 ±1.6	21.83 ±1.068	3.66
Diazepam	20 ±1.6	19.16 ±1	4.2
MEMA (250mg/kg)	16.16 ±1.4	17.16 ±0.66	6.18
MEMA (500mg/kg)	17.16 ±1.06	15.8 ±1.8	7.92

% reduction in Locomotor activity:(Wa-Wb)/Wa×100

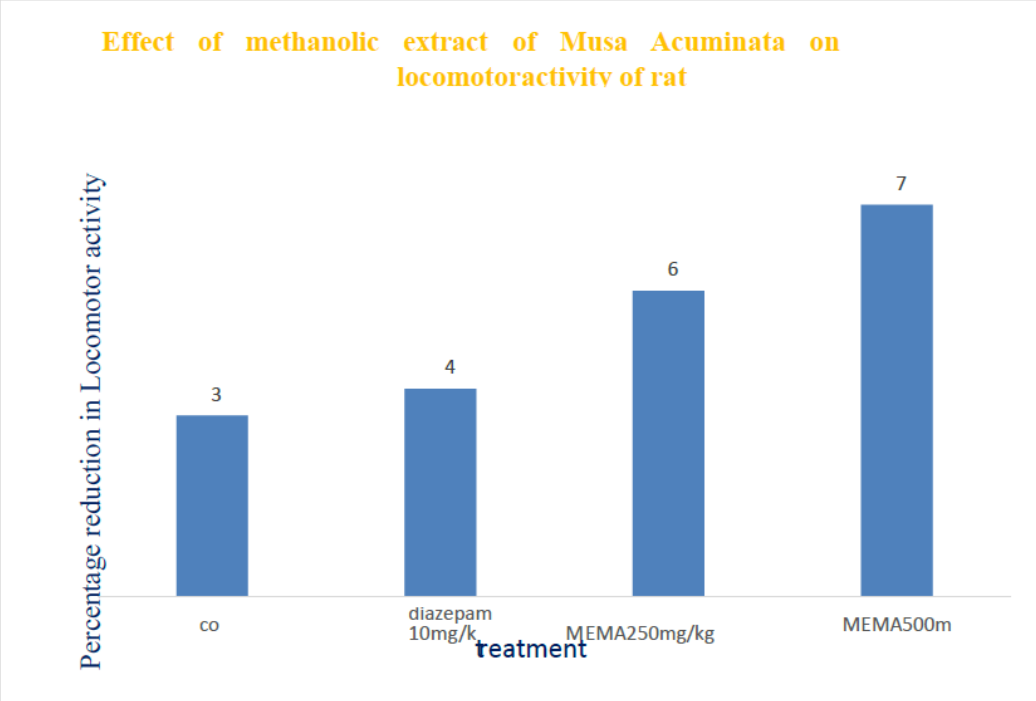
Where

Wa=actiphotometer score before treatment,

Wb= actiphotometer score after treatment.



Graph No: 11 effect of methanolic extract of *Musa Acuminata* on locomotor activity of rat



Graph-12 Effect of methanolic extract of Musa Acuminata on locomotoractivity of rat

In locomotor activity study it was found that MEMA significantly ($p<0.0005$) depressed the locomotor activity in a dose and time dependent manner. The activities increased as time approached to 60 min the results are summarized in table 18. the percentage of reduction in thelocomotor activity with diazepam (10mg/kg i.p) after 60 min was 4.2. percentage of reduction inthe locomotor activity with MEMA (250mg/kg &500mg/kg p.o) after 60 min was 6.18 and 7.92. That is there was a highly significant $p<0005$) decrease in locomotor activity compared to control as well as standard. Maximum muscle relaxation observed with 250mg/kg & 500mg/kg MEMA.

Statistical analysis -
Results were expressed as Mean± standard deviation, statistical analysis was performed using one way analysis of variance followed by Dunnet test. $P<0.0005$ was considered statistically significant

III. DISCUSSION

In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. *Musa Acuminata* is one of them and used against various disorders in indigenous system of medicine, especially for *Musa acuminata* has been traditionally used to treat various diseases and ailments such as fever, bronchitis, allergic reactions, sexually transmitted infections, and some non-communicable diseases.

The compounds isolated from *Musa acuminata* have been used as anti-hypertensive, anti- diabetic, anthelmintic, and anti-HIV; and have proven useful against tuberculosis and other respiratory diseases traditionally.

All parts of the plant including fruit, stem, pseudo stem, flower, leaf, sap, inner trunk, inner core, and root have found their use in traditional medicine. The objective of present study was too investigative the effect of methanolic extract of *Musa Acuminata* on muscle relaxant activity in experimental animals like Wistar albino rats. Muscle relaxant property *Musa Acuminata* extract was studied using Rotarod apparatus, and total fall off time for standard and control group was recorded.

Actiphotometer is widely used screening model for evaluating the locomotor activity and rotarod model for muscle relaxation. The extracts were subjected to phytochemical screening, for presence of carbohydrates, proteins, flavonoids, alkaloids, steroids, phenolics compound.

The reduction of spontaneous motor activity could be attributed to the sedative effect of the extract.

The present study showed a dose -dependent increase in muscle relaxation withdifferent doses of MEMA. Decrease in locomotion implies skeletal muscle relaxation effect oncentral nervous system.
The extracts were subjected to phytochemical screening, methanolic extract showed thepresence of carbohydrates, flavonoids, alkaloids, phenolics compound. The muscle relaxant activity observed may be due to presence of flavonoids, alkaloids, tannins and phenolics compounds.

The standard reference drug diazepam acts as an anxiolytic at low doses and a muscle relaxant at higher doses. Diazepam has been used in several studies as a positive control for testing the skeletal muscle relaxation. Diazepam is a centrally acting skeletal muscle relaxant which 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

In this study diazepam at the dose of 10mg/kg body weight showed a significant motorcoordination and muscle relaxant activity in rats. Rats treated with extract showed muscle relaxation and reduced motor activity. In rotarod motor & actiphotometer model motor coordination test at 250mg/kg & 500mg/kg show significant effect as compare to standard.

Further extensive phytochemical analysis and research is necessary to identify the exact constituents and elucidation of its possible mechanism of action underlying the myorelaxant activity of Methanolic extract of *Musa Acuminata.*

IV. SUMMARY AND CONCLUSION

In this modern world and confused lifestyle, the graph of disease is increasing. Due to this people are taking medicines which are having a lot of side effects and problems. So, the need of side effects free medications is rising. There is an increasing voice for side effects free medications. From old generations we have learnt that they used plants or their parts as medicine for various diseases. In many developing countries, most of the people prefer traditional system of medicine as their treatment.

India is a country with large environmental area, which gives us treat to explore the different plants with different species. We have the support of knowledge of Ayurveda. The natural ingredients in the plants with side effects free treatment are advantageous for us. Hence, we should work more towards the natural products.

Musa Acuminata commonly known as banana belong to Musaceae family. The literature survey exposed that there is no efficient and scientific study on skeletal muscle relaxant activity of Musa Acuminata pulp extract available. Therefore, it was thought useful to discover this plant for its skeletal muscle relaxant activity.

Three extracts of *Musa Acuminata were* prepared with methanol, acetone and water. The preliminary phytochemical investigation was carried out for all three extract to determine the phytoconstituent present in extract. The preliminary phytochemical valuation of plant extract tells the presence of flavonoid, carbohydrate, proteins, amino glycosides, phenolics, tannin, alkaloids, and saponin. These phytochemicals were confirmed by qualitative analysis.

The skeletal muscle relaxant activity of methanolic extract of *Musa Acuminata* fruit pulp was studied by using Rotarod apparatus and Actiphotometer model. The results were related with standard drug diazepam.

In Rotarod model, only those rats which had demonstrated their ability to remain on revolvingrod (20rpm) for 5min, were used for test.30 mins after the injection of test material and standard drug the rats were placed on the revolving rod and fall of time i.e. when rats fall from the rotating rod, was recorded, which was taken as grip strength. Due to administration of test and standard drug muscles of rat were relaxed and fall of time of the rat from the rotating rod weredecreased, which is taken as index of muscle relaxation.

Actiphotometer is another model used for skeletal muscle relaxation study, each animal is placed in square closed field area equipped with photocells and after 60 min of administration of test and standard drug, each rat is placed in Actiphotometer activity cage for 5 min, the difference in activity score before and after administration of drug was noted. From this percentage decrease in locomotor activity was calculated.

Methanolic extract of Musa Acuminata at the dose of 250mg/kg and 500mg/kg shows better locomotor activity. While methanolic extract pf Musa Acuminata at the dose of 250mg/kg and 500mg/kg shows better motor activity in Wistar rats as compared to control group.

But when associated with standard drug diazepam methanol extract of Musa Acuminata at 250and 500 mg/kg dose have very less difference in skeletal muscle relaxant activity. That is the methanol extracts at 250mg/kg & 500 mg/kg shows near similar activity like diazepam

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