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INVESTIGATION OF *IN- VIVO* ANALGESIC AND *IN-VITRO* THROMBOLYTIC ACTIVITIES OF HYDRO ALCOHOLIC LEAF EXTRACT OF *Musa balbisiana*

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ABSTRACT
The plant under investigation (*Musa balbisiana*) was a giant monocotoyledenous herbs and belongs to the family Musaceae. The goal of our investigation was to determine whether the leaf extracts of this plant held any significant medicinal properties. Leaves of *Musa balbisiana* were extracted with hydroalcohol. The extracts and fractions were tested for phytochemical analysis, analgesic activity was evaluated employing the eddy's hot plate and thrombolytic activity was evaluated by the clot lysis test. The extract of all the fractions and streptokinase (standard) exhibited significant clot lysis. The hydro alcoholic leaf extract and fractions produced significant analgesic effects as evaluated. In the hot plate method the extract produced a significant ($p < 0.001$) dose dependent reduction of thermally induced pain. The overall results suggested that this plant deserves further investigation to isolate the active compounds which are responsible for these activities and to establish the mechanism of action.

KEYWORDS: Phytochemical, *Musa balbisiana*, Eddy's hot plate, Thrombolytic activity.

INTRODUCTION
Although the introduction of scientific study on herbal medicines is new but the use of herbal medicines has been gifted as a blessing to the mankind for its fewer side effects. In history plants have been used for medicinal purposes prevent when all these advanced technologies were not introduced. In the early 3000 BC ancient Chinese and Egyptian papyrus used herbal medicines for the betterment of health. Different cultures used herbs in different aspects of treatment and diagnosis. Many herbal plants are used for health beneficial in different region of world.[1]

Plants have been one of the rich and important sources of medicines since the dawn of human civilization. These are the gift of nature to the mankind for treating different types of diseases. Almost from prehistoric period, use of herbal medicine for alleviation of suffering caused by different diseases in human are well documented in India and other countries and even today they are in great use in these countries.

In recent years, medical science has experienced dramatic changes, and surprisingly, every year the global traditional herbal medicine market is growing, and it is anticipated that within 2050 this market will reach to 5 trillion dollars.

Pain has been defined by International Association for the Study of Pain (IASP) as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Failure to relieve pain is morally and ethically unacceptable. All these drugs carry potential toxic effects. Pain, pyrexia and inflammation act as a warning of external noxious stimuli and microbial invasion to the body. However, they are viewed as sources of discomfort and are commonly suppressed with analgesic medications respectively.[6] These conventional drugs may have various severe side effects. The major adverse reactions of ibuprofen, an analgesic, include the effects on the kidney, the gastrointestinal tract and the coagulation system.[7] Diclofenac, an analgesic and anti-inflammatory drug, is a known hepatotoxic drug in certain individuals and it also causes deposition of urate crystals in kidneys, liver, heart and spleen.[8] Sulindac causes serious gastrointestinal (GI).adverse effects including inflammation, ulceration, bleeding, stomach perforations, large and small intestines perforations, which can be fatal.[9] In addition to having the above side effects, the conventional drugs are expensive and have low efficacy.[10] Piroxicam increased the risk of bleeding in both acute and chronic therapy.[11] Opioids are the commonly used drugs for the management of acute postoperative pain.[12] One study suggests that

risk of gastrointestinal bleeding was significantly associated with acute use of non-steroidal anti-inflammatory drugs (NSAIDs) like regular-dose aspirin, diclofenac, ketorolac, naproxen or nimesulide.

Thrombolysis stands out as the breakdown (lysis) of blood clots by just pharmacological means.[13] It is colloquially mentioned as clot busting for this evidence. It works by stimulating fibrinolysis by just plasmin through infusion of analogs affecting tissue plasminogen activator (tPA), one's protein that normally activates plasmin.[14] Thrombolysis suggests the usage of thrombolytic drugs, which are either that, is generated by Streptococcus species or more simply just lately, using recombinant biotechnology whereby tPA is usually manufactured by bacteria, resulting in a recombinant tissue plasminogen activator together with rtPA. Formation of blood clots is place for the basis of many serious disorders.[15] By extracting the clot, the disease process might possibly be arrested, or the complications receded.

Plantains and bananas are giant monocotoyledenous herbs. It is originated in Western Pacific and Southeast Asia. *Musa balbisiana* belongs to the family Musaceae is one of the most important species which is involved in the origin of cultivated bananas and it is distributed from India to Papua, New Guinea. Earlier it was believed that this species possessed only limited variability. However, recent works have shown M. balbisiana also contain good level of infraspecific variability. This therefore calls for continuous research to discover new compounds as therapeutic alternatives.[16] showed that naturally occurring agents, such as *M. balbisiana* derivatives are best alternatives.

Taxonomy
Kingdom: Plantae
Division: Angiospermae
Class: Scitaminae
Order: Zingiberales
Family: Musaceae
Genus: Musa

MATERIALS & METHODS

Plant material

The fresh leaves of *Musa balbisiana* belonging to the family Musaceae was collected from Penamaluru, Krishna District, Andhra Pradesh in September 2019.

Standard Drugs: Diclofenac, Streptokinase. These drugs were dissolved in distilled water. The solutions were freshly prepared just before the administration. All the drugs were administered orally through oral feeding syringe.

Experimental animals: Spargue dawley rats of either sex weighing between 100-200 grams were obtained from **Mahaveer Enterprises, Hyderabad,** which were maintained at Vijaya Institute of Pharmaceutical Sciences for Women, (Vijayawada, India) at standard conditions. They were housed in well- ventilated cages maintained at 25±2^{0}C, with 12 hours dark/ light cycle. They were fed with standard pellet diet supplied by **Sai Durga Feeds and Foods, Bangalore,** and had free access to water except just before and during experimentation. The animals were maintained in these conditions for one week before the experimental session. Our Institutional Animal Ethics Committee (IAEC) has approved this study.

Collection and Drying

The fresh leaves of *Musa balbisiana* were washed and left for shade drying for 15 days. The leaves were powdered.

Extraction Procedure

In this study soxhlet extraction procedure was employed with the mixture of equal volume of water (200 ml) and ethanol (200 ml). About 200 g leaves powder was taken into paper cylinder made from whatman filter paper and is placed in the body of the soxhlet extractor. The solvent is placed in the flask in equal ratio of water and ethanol. The soxhlet with its contents was sealed and kept for a period of 36 hours for soxhalation. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through Whatman 41 filter paper. The solvent was completely removed by and crude extract was dried in waterbath. This crude extract was used for investigations.

Macroscopic evaluation: Organoleptic evaluation of the powdered plant was carried out using standard methods.[15] Sensory organs were used to evaluate the colour, odour, taste and texture of the plant powder.

Phytochemical analysis: The phytochemical screening was carried out on the crude extract and fractions of *Musa balbisiana* leaves according to standard methods to identify the classes of bioactive compounds present.

Acute toxicity study

Acute oral toxicity study was performed as per OECD 423 guidelines (acute toxic class method), Sprague Dawley rats of weight 180-220 gm (n = 3) of either sex selected by random sampling were used for acute toxicity study. The animals were kept fasting overnight and provided only with water, after which the tract was administered orally at 1000, 2000 & 5000 mg/kg and observed for 14 days. If mortality is observed as two out of three animals, then the dose administered is assigned as a toxic dose. If mortality observed in one animal, then the same dose is repeated to confirm the toxicity study.

Determination of analgesic activity by Eddy's hot plate method: Rats weighing 150-180 g were used. Rats were placed on the hot plate, which consists of electrically heated surface. Temperature of the hot plate was maintained at 55°C. Responses such as jumping, withdrawal of the paws and licking of the paws were

observed. The time period (latency period) when animals were placed and until responses occur was recorded by the stopwatch. Extracts was administered orally and latency period was recorded after 0, 30, 60, 90 and 120 min. These values were compared with the standard drug and control normal saline. This model evaluates the central pain.

Grouping of animals: Each group consist of 6 rats of either sex.
Group 1: Control group, treated with normal saline.
Group 2: Treated with *M. balbisiana*, (200mg/kg) a test drug on the day of the experiment.
Group 3: Treated with *M.balbisiana*, (400mg/kg) a test drug on the day of the experiment.
Group 4: Treated with Diclofenac, a standard drug (10mg/kg) on the day of the experiment.

Assessment of Thrombolytic Potential: A method developed by Prasad *et al*., was used for the assessment of the *in-vitro* thrombolytic activity of *Musa balbisiana* extract using Streptokinase (at 15000 and 30000 I.U) as a positive control with minor modifications [21]. 5 ml blood had drawn from healthy volunteers (n = 3) and transferred to the micro-centrifuge tube (1 ml/tube) for incubation for 45 min at 37 °C. After clot formation, measured the weight and 100 µl of the plant extract with various concentrations (2, 4, 6, 8 & 10 mg/ml) suspended overnight was added to the tubes accordingly. 100 µl of streptokinase as a positive control and 100 µl of sterilized distilled water as a negative non-thrombolytic control were added to the control tubes. All tubes were incubated again for 90 min at 37 °C and observed for clot lysis. Finally, the differences in weight taken before and after clot lysis were expressed as a percentage of clot lysis following the under beneath equation.

% of clot lysis = Wt. of released clot/clot wt. × 100
$= W_2 - W_3 / W_2 \times 100$
Here, W_2 = Weight of clot after 45 min incubation (gm), W_3 = Weight of lysised clot after 90 min incubation (gm).

Statistical Analysis: The data obtained by various parameters were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA). $P < 0.001$ was considered stastically significant.

RESULTS

Phytochemical Investigation

Table 1: Phytochemical Investigation of *Musa balbisiana* (Leaves).

S.No	Test	Presence
1.	Carbohydrates	+
2.	Proteins	-
3.	Aminoacids	-
4.	Steroids	-
5.	Fixed Oils & Fats	-
6.	Flavanoids	+
7.	Glycosides	-
8.	Alkaloids	-
9.	Tannins & Phenolic Compounds	+

(+) indicate positive means present
(-) indicate negative means absent

Result of Acute toxicity test of hydroalcoholic leaf extract of *Musa balbisiana*

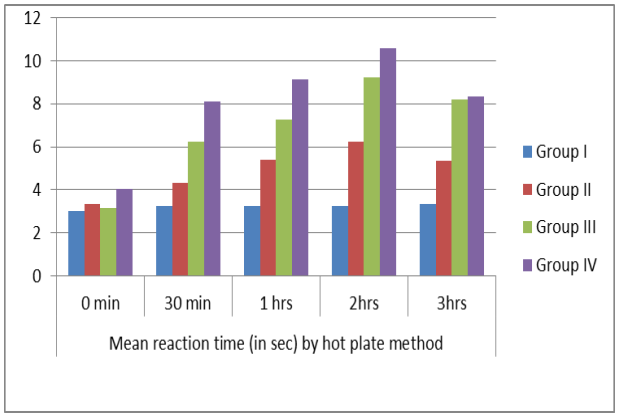
The fasted animals used in the first phase of the test were observed to be visibly calm after oral administration. No visible signs of pain or discomfort were observed. From the toxicity study, it was observed that the powdered leaves of *Musa balbisiana* leaf was non-toxic and caused no death up to 5000mg/kg orally. Toxicological studies established LD50 of the crude to be greater than 5000mg/kg showing it is safe for consumption.

Result of Analgesic activity by Eddy's hot plate Method: The effect of extracts on analgesic activity by eddy's hot plate was showed in table 5.2. The oral administration of *Musa balbisiana* (200 & 400 mg/kg) respectively caused significant prolongation of the reaction time after 30 minutes administration when compared to control group. The prolongation in the reaction time showed central analgesic activity of the extracts. The antinociceptive response of both the extracts was tabulated in 5.2 when compared to standard drug Diclofenac (10 mg/kg). The response of the hydro alcoholic extract (200 & 400 mg/kg) on hot plate was shown in graph 5.1. It was observed that oral administration of extracts respectively produced significant dose dependent analgesic effect.

Table 2: Analgesic activity of Hydro alcoholic Extract of *Musa balbisiana* by Eddy's hot Plate Method.

Groups	Dose	Mean reaction time (in sec)				
		0 min	30 min	1 hrs	2 hrs	3 hrs
Group I	Saline	3.03±0.151	3.23±0.075	3.22±0.074	3.24±0.076	3.34±0.071
Group II	200 mg/kg	3.34±0.071	4.31±0.076**	5.38±0.07***	6.25±0.147***	5.34±0.205***
Group III	400 mg/kg	3.14±0.181	6.22±0.144***	7.19±0.153**	9.23±0.143***	8.21±0.232**
Group IV	10 mg/kg	4.04±0.216	8.10±0.177***	9.14±0.186***	10.60±0.289**	8.36±0.196***

Value expressed as Mean ± SEM; n=6 animals in each group
$P < 0.001$ was considered statistically significant.



Graph 1: Analgesic activity of Hydro alcoholic Extract of *Musa balbisiana* by Eddy's hot Plate Method.

Assessment of Thrombolytic Potential: The *in-vitro* thrombolytic activity of *Musa balbisiana* extracts using Streptokinase (at 15000 and 30000 I.U) as a positive control. 5 ml blood had drawn from healthy volunteers (n = 3) and transferred to the micro-centrifuge tube (1 ml/tube) for incubation for 45 min at 37 °C. After clot formation, measured the weight and 100 µl of the plant extract with various concentrations (20 & 40 mg/ml) suspended overnight was added to the tubes accordingly. 100 µl of streptokinase as a positive control and 100 µl of sterilized distilled water as a negative non-thrombolytic control were added to the control tubes. All tubes were incubated again for 90 min at 37 °C and observed for clot lysis. Finally, the differences in weight taken before and after clot lysis were expressed as a percentage of clot lysis following the under beneath equation.

% of clot lysis = Wt. of released clot/clot wt. × 100

$= W_2 - W_3 / W_2 \times 100$

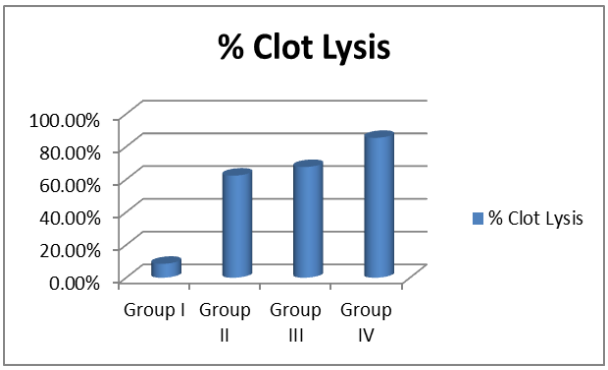
Table 3: Thrombolytic activity of Hydro alcoholic Extract of *Musa balbisiana*.

Groups	Drug	Dose	% Clot Lysis
Group I	Control	Water	8.5%
Group II	*M.balbisana* Extract	20 mg/ml	62.35%
Group III	*M.balbisana* Extract	40 mg/ml	67.61%
Group IV	Streptokinase	3000 units	85.41%

The percentage of fat decrease of clot after application associated with extract solution was taken because functional indication of thrombolytic exercise. The average value of percentage of fat loss was calculated and shown upon table. The percentage of clot lysis of standard was 85.41%.

The sample hydro alcoholic extract of *M.balbisiana* leaf extract shows strong percentage of clot

Lysis is 62.35%, 67.61%. Actually simply leaves have significant thrombolytic activity. Numerous plants source especially several fruits and vegetables have been studied for their own supplements having anticoagulant, antiplatelet and fibrinolytic activity and there might be evidence that consuming such food results in prevention connected with coronary occasions and stroke.



Graph 2: Thrombolytic activity of Hydro alcoholic Extract of *Musa balbisiana*.

DISCUSSION

The present study was aimed to study the analgesic and thrombolytic activities of hydro alcoholic extract of *Musa balbisiana* leaves. The preliminary phytochemical analysis of extract showed the presence of flavanoids, tannins and phenols. The hot plate model has been used for the study of centrally acting analgesia (antinociception). Here the nociceptors seem to be sensitized by sensory nerves and the involvements of endogenous substances such as prostaglandins (PGs) are minimized. In the hot plate models, the tolerance to thermal stimulus manifested by increase in the pain reaction time indicates the level of antinociception induced by extract or drug. The oral administration of *Musa balbisiana* (200 &400 mg/kg) respectively caused significant prolongation of the reaction time when compared to control group. The response of extracts on Eddy's hot plate method showed significant dose dependent analgesic effect at all test doses when compared to control and standard.

A blood clot (thrombus) developed in the circulatory system due to failure of hemostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death. Thrombolytic therapy is used to dissolve blood clots that could cause serious, and possibly life-threatening, damage if they are not removed. The *Musa balbisiana* hydro alcoholic leaf extract exerted 62.35% & 67.61% from the blood clot in the thrombolytic activity while standard and control acquired 85.41% & 8.5%.

CONCLUSION

Herbal medicines are produced from different parts of particular plants, for example- seeds, roots, stems, barks, leaves, berries etc. The components determined by the phytochemical screening of the hydroalcoholic leaf extract of *Musa balbisiana* showed the presence of various valuable chemicals like- flavanoids, tannins and phenols etc. In the present study, the hydroalcoholic leaf extract of *Musa balbisiana* clearly exhibited the analgesic activities in experimental animal models. It can be used as a therapeutic agent in the treatment of pain as it posses centrally acting analgesic effect. Further studies shall be undertaken for elucidating the exact mechanism of action of the plant extracts in reducing pain. Thrombolytic study of *M. balbisiana* shows that it has thrombolytic activity which was good but using different isolation techniques the constituents could be separated and further investigation on thrombolytic activity can be done.

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