Volume 11, Issue 1, January – 2026 International Journal of Innovative Science and Research Technology
ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

Antipyretic and Analgesic Activity of *Dioscorea bulbifera* on Wistar Albino Rats – Three Arm Randomized Control Trial

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Publication Date: 2026/02/04

Abstract: Fever, or pyrexia, is defined as an elevation in body temperature beyond normal physiological limits and pain an unpleasant sensory and emotional experience occur in some types of fever. *Dioscorea bulbifera* leaves are traditionally used for managing fever with aches and pain as per Siddha Literature. Based on these traditional claims, the present study aimed to scientifically evaluate the antipyretic and analgesic activities of *Diacorea bulbifera* leaf decoction using experimental animal models. For antipyretic activity, 18 male Wistar albino rats were divided into three groups: control, standard, and test. Fever was induced using 15% w/v Brewer's yeast administered subcutaneously. After 18 hours, rectal temperatures were recorded at 30, 60, 90, and 120 minutes following treatment. The control group received 1 ml/kg normal saline, the standard group received paracetamol syrup, and the test group received *Diacorea bulbifera* leaf decoction according to standard dose calculation. For analgesic activity, 18 female Wistar albino rats were divided similarly, and the tail immersion method was used. Tail withdrawal times were recorded at 30, 60, 90, and 120 minutes after administering saline, diclofenac and diascorea *bulbifera* decoction. The *Dioscorea bulbifera* decoction significantly reduced fever, showing effects comparable to paracetamol after 90 minutes. It also exhibited notable analgesic activity from the 60th minute onward, with stronger and longer-lasting effects than diclofenac. Dioscorea bulbifera leaf decoction demonstrates significant antipyretic and analgesic effects, supporting its traditional use in treating fever and pain.

Keywords: *Antipyretic, Analgesic, Decoction, Wistar Albino Rats, Rectal Temperature.*

How to Cite: A. S. R. Perera; N. Varnakulendran (2026) Antipyretic and Analgesic Activity of Dioscorea bulbifera on Wistar Albino Rats – Three Arm Randomized Control Trial. *International Journal of Innovative Science and Research Technology*, 11(1), 2659-2665. https://doi.org/10.38124/ijisrt/26jan1327

I. INTRODUCTION

Elevation of the body temperature than normal level sometime increase temperature may occur in the part of the body that called "Suram". Suram may be associated with burning sensation of eye, nausea, vomiting, headache, heaviness of the head [1,2]. It compares with fever in modern medicine. A fever is usually a sign that something out of the ordinary is going on in the body. For an adult, a fever may be uncomfortable, but fever usually is not dangerous unless it reaches 103º F (39.4ºC) or higher. For very young children and infants, a slightly elevated temperature may indicate a serious infection. But the degree of fever does not necessarily indicate the seriousness of the underlying condition. A minor illness may cause a high fever, and a more serious illness may cause a low fever [3,4]. Fever can be caused by many medical conditions such as viral bacterial and parasitic infections such as the common cold, urinary tract infections, meningitis, malaria and appendicitis. Noninfectious causes include vasculitis, deep vein thrombosis, Side effects of medication, and cancer among others [5].

The international association of study of pain definition states "pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. Pain is a major symptom in many medical conditions and significantly interferes with a person's quality of life and general functioning [6,7]. The modern drugs (opioids, NSAIDs, corticosteroids) currently used for the management of pain, fever and inflammatory conditions, present with many known adverse

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

effects. Moreover, synthetic drugs are expensive, and many medicinal herbs have been used as therapy for the relief of pain without much adverse effects [8].

Dioscorea bulbifera from *Dioscoreaceae* family is widely used by the traditional medical practitioners for curing various diseases in their day-to-day practice. As well as it composes anti-pyretic and analgesic activity of dry leaves of *Dioscorea bulbifera* which has not been proved scientifically so far. Therefore, this research was conducted to prove the antipyretic and analgesic activity in scientific method. *Dioscorea bulbifera* dry leaves is enhanced anti-pyretic and analgesic activity which is mentioned in the text book *Kunapadam* Part-1 (Porut Panpiyal) written by *Murugesamuthaliyar*, it was stated that the plant is applicable for *suram* and *soolai*.

Plant *Dioscorea bulbifera* belongs to the family *Dioscoreaceae*. Plant has been under went many researches. Use for various medical conditions. According to siddha text leaves, flowers, fruit, seeds, oil, bark, dry leaves are the commonly using parts. Plant is known as *Kaai vallikodi* in tamil and found throughout India and Sri Lanka. The bark of this plant is a good remedy for itch, swellings, fractures and snake bite poisoning. The oil extracted from the seed is applied on swellings, rheumatism and other skin diseases. The heartwood made into a paste is applied on the throat for glandular swellings in the neck and throat [9,10]. Contains chemical constituents such as Alkaloids, glycosides, proteins, fats, sterols, polyphenols, saponin, flavonoids, and tannins. Other than anti-pyretic and analgesic activities, the plant contains antiulcer, antimicrobial, antioxidant, hepatoprotective, anti-inflammatory and wound healing property [11,12].

II. MATERIALS AND METHODS

➢ *Study Design*

Three arm Randomized Control Trials.

➢ *Study Population*

For anti-pyretic activity - 18 wellbeing male rats with 150-200g. For analgesic activity - 18 wellbeing female rats with 150-200g. Healthy female Wistar albino rats weighing 150-200g was obtained from Medical Research Institute (MRI) animal house for experimental study. Wellbeing female rats, Normal behavior and activity, 150 - 200g weight rats were included. Diseased rats and weight were above or below 100g-200g with abnormal behavior and activity were excluded. The animals were kept in aluminum cages with not more than six animals per cage in a room maintained under standard controlled atmospheric conditions. They were allowed free access to standard dry pellet as basal diet and water ad libitum. The rats were acclimatized to laboratory conditions for 10 days before commencement of the experiment [15].

➢ *Grouping of Animals*

For antipyretic activity, animals were divided into 3 groups. Each group contained 6 animals. Group I - was treated orally with 1ml sterile normal saline with a syringe in the morning and resemble as the control group. Group II - was treated with paracetamol 13 drops with syringe in the morning and resemble as the standard drug. Group III - was treated with tested drug 9 drops with syringe in the morning and resemble as test group (*Dioscorea bulbifera* dry leaves decoction).

For analgesic activity, group I - was treated orally with 1ml sterile normal saline with a syringe as the control group. Group II - was treated orally with 3.3 mg Diclofenac with a syringe as the standard group. Group III - was treated orally with 9 drops of *Dioscorea bulbifera* dry leaves decoction as the test group.

➢ *Selection of the Plant*

The plant *Dioscorea bulbifera* was selected from the Siddha text named, Siddha Materia Medica by Dr Murugesamudhaliyar., page no:218 [13]. It was collected near the house area, Ruwanwella, Kegalle District, Sri Lanka and authenticated by Department of Gunapadam (Siddha Pharmacology), Faculty of Siddha Medicine, Trincomalee Campus, EUS.

➢ *Preparation of Dry Leave Decoction of Dioscorea Bulbifera*

The *Dioscorea bulbifera* plants were collected from house garden, Ruwanwella. The collected leaves were washed thoroughly in the tap water and allowed the water to drain off. Then dried it for puriication. After that made into a decoction according to the general rule of decoction preparation method and stored in a clean air tight container. Leaves of *Dioscorea bulbifera* - 1 part, Add water - 12 parts, Reduced water in to 1/12 [14].

➢ *Procedure for Antipyretic Activity*

The 15% (w/v) suspension of Brewer's yeast solution was prepared weighed 7.5g and it was mixed with 50ml of 0.9% saline. To use as a dose for induced fever 10ml/kg (2ml/200mg) (15g yeast mixed with 100ml of water) [19]. Female wistar rats of with body weight 150-200g were used. Then divided into 3 groups. Each group compromising 6 rats and cages were labelled. By insertion of thermo couple in the depth of 2cm in the rectum the initial rectal temperature was recorded. Before it, the animals are fevered by injection of 10ml/kg (2ml/200mg) of brewer's suspension subcutaneously in the back below the nape of the neck of wistar rats and immediately massaged on injected area. Food is withdrawn. 18 hours post challenge, the raise in rectal temperature was recorded. The measurement was repeated after 30 minutes. Only animals with the body temperature of at least 38ºC are taken in to the test. After animal received the test drug or the standard drug by oral administration, the rectal temperatures were recorded again in 30, 60, 120 and 180 minutes.

➢ *Procedure for Analgesic Activity*

The animals were screened for sensitivity to heat prior to the analgesic experiment. Hence the tails of the animals would be immersed into the hot water for 10 seconds and Selected for

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

the study. The evaluation will be done by immersing the lower 5cm portion of the tall into beaker of water maintained at 55± 0.5 ºC. The time in seconds for tail withdrawal from the water was took as the reaction time with a cut-off time of immersion at 10 seconds. The reaction time was measured before and after drug administration and 30, 60, 90, 120 minutes [20]. Procedure was done to both standard and test drugs.

➢ *Data Analysis*

The data was entered, coded, and analyzed using statistical package for the social sciences (SPSS). Statistical analysis was done by Descriptive, Tukey's Honest Significant Difference (HSD) test and one-way analysis of variance (ANOVA) by using IBM, SPSS Version 28 for the circumference of wound and p value < 0.05 was considered statistically significant.

III. RESULTS

In this study, the rectal temperatures of all animal before induce of fever, after 18 hours of inducing of 15% Brewer's solution according to the rats' weight fever were measured. Then normal saline was administrated for control group, paracetamol syrup for standard group and *Kaai vallikodi* dry leaves decoction for test group. Then rectal temperatures were measured after 30th minute, 60th minute, 90th minute, 120th and 150th minute intervals after administration of drug.

➢ *Antipyretic Activity*

Table 1: Descriptive Temperature Analysis of all Three Groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Initial Temperature								
Control	6	98.5333	.59554	.24313	97.9084	99.1583	97.90	99.50
Standard	6	99.2000	.47749	.19494	98.6989	99.7011	98.60	99.70
Test	6	99.3333	.49666	.20276	98.8121	99.8545	98.50	99.80
Total	18	99.0222	.61122	.14407	98.7183	99.3262	97.90	99.80
After 18 hour of inducing pyrexia								
Control	6	102.8333	.91141	.37208	101.8769	103.7898	101.50	103.90
Standard	6	101.9833	.35449	.14472	101.6113	102.3554	101.60	102.60
Test	6	102.8667	.28048	.11450	102.5723	103.1610	102.60	103.40
Total	18	102.5611	.69379	.16353	102.2161	102.9061	101.50	103.90
30 minutes (ºF)								
Control	6	102.8167	.95167	.38852	101.8180	103.8154	101.40	103.80
Standard	6	101.7333	.24221	.09888	101.4791	101.9875	101.40	102.00
Test	6	102.1000	.23664	.09661	101.8517	102.3483	101.80	102.40
Total	18	102.2167	.71723	.16905	101.8600	102.5733	101.40	103.80
60 minutes (ºF)								
Control	6	102.9167	.90646	.37006	101.9654	103.8679	101.60	104.00
Standard	6	101.1667	.81650	.33333	100.3098	102.0235	100.10	102.00
Test	6	101.7667	.27325	.11155	101.4799	102.0534	101.40	102.10
Total	18	101.9500	1.00893	.23781	101.4483	102.4517	100.10	104.00
90 minutes (ºF)								
Control	6	102.9500	1.19610	.48831	101.7781	104.2886	101.20	104.60
Standard	6	100.5833	1.19569	.48814	99.3285	101.8381	99.30	102.30
Test	6	100.7667	.71740	.29288	100.0138	101.5195	99.80	101.80
Total	18	101.4611	1.51895	.35802	100.7058	102.2165	99.30	104.60
120 minutes (ºF)								
Control	6	102.9800	.68823	.28097	102.6611	104.1056	102.50	104.50
Standard	6	98.200	.98995	.40415	96.8611	98.9389	96.50	99.10
Test	6	98.9000	1.22147	.49866	97.6181	100.1819	97.50	100.20
Total	18	100.0611	2.62413	.61851	98.7562	101.3661	96.50	104.50
150 minutes (ºF)								
Control	6	102.9333	.87788	.35839	102.0121	103.8546	101.50	104.00
Standard	6	98.2667	.52789	.21551	97.7127	98.8207	97.60	99.00
Test	6	98.8000	.67750	.27659	97.4390	98.8610	97.10	99.00

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

Total	18	99.7833	2.38728	.56269	98.5962	100.9705	97.10	104.00

Table 2: Inter Group Analysis of Mean Temperature

Time Point / Condition	Source of Variation	Sum of Squares	df	Mean Square	F Value	Sig. (p)
Initial Temperature	Between Groups	2.204	2	1.102	3.987	0.081
	Within Groups	4.147	15	0.276	-	-
	Total	6.351	17	-	-	-
After 18 Hours (Induced Pyrexia)	Between Groups	3.008	2	1.504	4.359	0.052
	Within Groups	5.175	15	0.345	-	-
	Total	8.183	17	-	-	-
30 Minutes	Between Groups	3.643	2	1.822	5.356	0.018
	Within Groups	5.102	15	0.340	-	-
	Total	8.745	17	-	-	-
60 Minutes	Between Groups	9.490	2	4.745	9.107	0.003
	Within Groups	7.815	15	0.521	-	-
	Total	17.305	17	-	-	-
90 Minutes	Between Groups	22.348	2	11.174	9.932	0.002
	Within Groups	16.875	15	1.125	-	-
	Total	39.223	17	-	-	-
120 Minutes	Between Groups	102.334	2	51.167	52.111	0.000
	Within Groups	14.728	15	0.982	-	-
	Total	117.063	17	-	-	-
150 Minutes	Between Groups	89.343	2	44.672	88.850	0.000
	Within Groups	7.542	15	0.503	-	-
	Total	96.885	17	-	-	-

Table 3: Mean Temperature Deviation of all three groups with the time

Group	Initial temperature	After 18 hour of inducing pyrexia	After 30 minutes	After 60 Minutes	After 90 Minutes	After 120 Minutes	After 150 Minutes
Control	98.53	102.83	102.81	102.91	102.95	102.9	102.93
Standard	99.2	101.98	101.73	101.16	100.58	98.92	98.26
Test	99.33	102.86	102.1	101.76	100.76	98.9	98.8
Total	99.02	102.56	102.21	101.95	101.46	100.06	99.78

- *Analgesic Activity*

Table 4: Descriptive Withdrawal Time Analysis of all Three Groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Before drug administration								
Control	6	1.9567	.48944	.19981	1.4430	2.4703	1.52	2.78
Standard	6	1.9633	.51991	.21225	1.4177	2.5089	1.26	2.69
Test	6	1.5617	.53286	.21754	1.0025	2.1209	.99	2.30
Total	18	1.8272	.52039				.99	2.78
After 30 minutes								
Control	6	1.9150	.42637				1.48	2.55
Standard	6	2.1667	.47610	.19437	1.6670	2.6663	1.50	2.80
Test	6	1.5250	.44752	.18270	1.0554	1.9946	1.01	2.02
Total	18	1.8689	.50281				1.01	2.80
After 60 minutes								
Control	6	1.9200	.42704				1.50	2.56
Standard	6	2.3667	.45019	.18379	1.8942	2.8391	1.80	3.00
Test	6	1.5767	.52694	.21512	1.0237	2.1297	1.01	2.33
Total	18	1.9544	.55288				1.01	3.00

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After 90 minutes								
Control	6	1.9050	.40303				1.51	2.50
Standard	6	2.5667	.45019	.18379	2.0942	3.0391	2.00	3.20
Test	6	2.1500	.59330	.24221	1.5274	2.7726	1.50	3.00
Total	18	2.2072	.53843				1.50	3.20
After 120 minutes								
Control	6	1.8850	.46436	.18957	1.3977	2.3723	1.30	2.55
Standard	6	2.4667	.45019	.18379	1.9942	2.9391	1.90	3.10
Test	6	1.9150	.49209	.20089	1.3986	2.4314	1.35	2.54
Total	18	2.0889	.51958	.12247	1.8305	2.3473	1.30	3.10

Table 5: Inter Group Analysis of Mean Withdrawal Time

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Before drug administration					
Between Groups	.635	2	.317	1.200	.329
Within Groups	3.969	15	.265	-	-
Total	4.604	17	-	-	-
After 30 minutes					
Between Groups	1.254	2	.627	3.091	.075
Within Groups	3.044	15	.203	-	-
Total	4.298	17	-	-	-
After 60 minutes					
Between Groups	1.883	2	.941	4.262	.034
Within Groups	3.313	15	.221	-	-
Total	5.196	17	-	-	-
After 90 minutes					
Between Groups	1.343	2	.671	2.809	.092
Within Groups	3.585	15	.239	-	-
Total	4.928	17	-	-	-
After 120 minutes					
Between Groups	1.287	2	.644	2.923	.085
Within Groups	3.302	15	.220	-	-
Total	4.589	17	-	-	-

Table 6: Mean Withdrawal Time Deviation of all Three Groups with the Time

Group	**Before drug administration**	**After 30 minutes**	**After 60 Minutes**	**After 90 Minutes**	**After 120 Minutes**
Control	1.9567	1.9150	1.9200	1.9050	1.8850
Standard	1.9633	2.1667	2.3667	2.5667	2.4667
Test	1.5617	1.5250	1.5767	2.1500	1.9150
Total	1.8272	1.8689	1.9544	2.2072	2.0889

IV. DISCUSSION

➢ *Antipyretic Activity*

The temperature in the Control group stabilizes and remains relatively consistent after the initial increase. The Standard group shows a distinct pattern: a sharp rise in temperature after 18 hours, followed by a gradual and steady decrease over the subsequent time points. In initial Temperature All three groups (Control, Standard, and Test) start at a similar baseline temperature, close to 99°F. This suggests that before the induction of pyrexia, the rats had no significant differences in body temperature across the groups.

After 18 Hours The temperature rises sharply in the Standard and Test groups, peaking at around 102°F. The Control group shows a much smaller increase, indicating that the fever induction (likely through a pyrogenic agent) was effective, and the antipyretic treatment had not yet been administered or taken effect. The Standard group shows a decrease in temperature, though it remains elevated. The Test group, also shows a temperature decrease, but slightly less than the Standard group. The Control group's temperature remains stable and slightly elevated, indicating no intervention was applied.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

60 Minutes After Treatment, Both the Standard and Test groups show further decreases in temperature. The Standard group has a sharper decline, suggesting that the conventional antipyretic might be more effective initially. The Test group also shows a reduction but at a slower rate, indicating the potential antipyretic action. The Control group's temperature remains stable. The trend continues with both the Standard and Test groups showing further reductions in temperature. By this time, the difference between the two treatments (Standard and Test) is becoming less pronounced, which might suggest that standard is catching up in its antipyretic effect. The Control group still shows minimal temperature change.

The temperature in the Standard and Test groups continues to decline, approaching normal levels. The gap between the Standard and Test groups narrows even further, suggesting that *Dioscorea bulbifera* might have a cumulative or delayed antipyretic effect compared to the standard treatment. The Control group remains relatively unchanged, confirming the lack of treatment.

Dioscorea bulbifera dry leave decoction lowered the elevated temperature more from 120th minute (2 hours) than in the 60 minute (1 hour) and the 30th minute. This might be attributed to the fact that most of the bioactive compounds may have not been completely absorbed across the gastrointestinal tract while in the 60" minute and most of the bioactive compounds may have been absorbed across the gastrointestinal tract from 90th minute thereby causing higher antipyretic activities. Lower percentage inhibition in the initial 30th minute could also be attributed to the fact that the drug needed time to be bio transformed into an antipyretic agent [21,22]. From the data obtained in this study, *Diacorea bulbifera* leaves decoction shows statistically significant differences from standard and control groups, as indicated by progressively lower p-values. And the test drug's effect becomes more pronounced as time progress.

- *Analgesic Activity*

Mean withdrawal time, shows steady in control group all over the 120 minutes. The mean withdrawal time gradually increase and then stabilizes with a slight decline toward the end. The mean withdrawal time gradually increase after 60th minute up to 90th minute and then gradually decrease up to 120th minute.

The control group shows relatively stable mean withdrawal times across the various time points, as expected. Since normal saline has no analgesic properties, the withdrawal times remain consistently low, indicating the rats' normal response to pain.

In standard drug, Initially, there's a slight increase in withdrawal time at the 30-minute mark, with a further increase up to the 90-minute mark, after which the effect slightly diminishes by the 120th minute. This pattern is typical for NSAIDs like diclofenac, which generally reach peak analgesic effect around 60-90 minutes post-administration. The gradual decline afterward may indicate the drug's diminishing efficacy as it is metabolized and excreted from the body [23].

In test drug group, notably there is an increase in withdrawal time starting at the 60-minute mark, peaking at 90 minutes, and then slightly decreasing by the 120th minute. This indicates that the test substance has a delayed onset of analgesic action compared to diclofenac but does eventually reach a comparable level of effectiveness. The decline at 120 minutes suggests that the analgesic effect is temporary and diminishes over time, similar to the standard drug, though with a delayed peak.

The differences in timing for the peak analgesic effect between the diclofenac and *Dioscorea bulbifera* groups could be attributed to differences in the pharmacokinetics of the substances. Diclofenac is known to be rapidly absorbed and metabolized, leading to quicker onset and offset of action. On the other hand, the active compounds in *Dioscorea bulbifera* might be absorbed more slowly or have different metabolic pathways, resulting in the delayed peak effect [24].

Diclofenac primarily works by inhibiting cyclooxygenase (COX) enzymes, reducing prostaglandin synthesis, which is responsible for pain and inflammation. The active compounds in *Dioscorea bulbifera* may have a similar but not identical mechanism, potentially involving additional pathways or receptor sites, which could explain the variation in time to peak effectiveness [23].

After the 60 minutes of drug administration, the p value is 0.034, means statistically significant. The descriptive statistics highlight that the Test group consistently has lower means than the other groups, particularly in after 60 minutes, which was found to be statistically significant. This suggests that whatever intervention or condition the Test group represents may have had a significant effect in reducing the outcome measured by after 60 minutes. The dry leaves decoction might have lower bioavailability compared to diclofenac, requiring more time to achieve sufficient plasma concentrations to exert an analgesic effect. Factors such as the method of preparation, the concentration of active ingredients, and the administration route can influence bioavailability [25].

V. CONCLUSION

The study concludes the antipyretic and analgesic activity of *Dioscorea bulbifera* leaves decoction on Wistar albino rats. The decoction demonstrated significant antipyretic activity (p 0.018), comparable to paracetamol, with effects becoming more pronounced over time. It also exhibited potent analgesic activity (p 0.034), when comparing to diclofenac in terms of onset and short duration of action. ($p < 0.05$) It is concluded that *Dioscorea bulbifera* dry leaves decoction is an effective in treatment for fever and pain, offering a valuable alternative to conventional medications.

ISSN No:-2456-2165 https://doi.org/10.38124/ijisrt/26jan1327

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