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An experimental study to evaluate galactagogue activity of *Bidali* (*Dioscorea pentaphylla* Linn.) with special reference to *Stanya Janana*

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ABSTRACT

More than 8 Lakh infantile deaths in India are attributed to inadequate breastfeeding, which is correlated with a prevalence of 45% to 53.6% cases of oligogalactia. *Bidali* (*Dioscorea pentaphylla* Linn.) is one among the two varieties of *Vidari* as described by *Acharya Dalhana,* with *Stanya Vardhaka Karma*. Hence the experimental evaluation of galactagogue activity of *Bidali (Dioscorea pentaphylla* Linn.) was carried out using milk estimation by the means of pup weight and weight gain along with estimation of serum prolactin and histology of breast tissue. Assessment was done by dam weight, daily milk yield by the means of pup weight and weight gain, serum prolactin estimation and histology of breast issue. The treatment groups have shown positive result in dose dependent manner in the parameters; Weight of Dams, and Weight of Pups. Histology reports re-emphasize the same; the development of Tubulo-alveolar unit and intraluminal secretions were more in group treated with higher dose than that of group treated with lower dose of *Bidali* (*Dioscorea pentaphylla* Linn.).

Key words: *Bidali, Dioscorea pentaphylla Linn., Galactagogue, Stanya Janana.*

INTRODUCTION

Inadequate breastfeeding contributes to a prevalence of 45% to 53.6% incidences of Oligogalactia, which is responsible for about 8 Lakhs deaths in India.[1] Despite many medications used in contemporary medicine claim to be beneficial, but they may also have long-term negative effects. For instance: Several gastrointe-stinal issues, insomnia, severe depression, and seizures are all brought on by the Metoclopramide (Dopamine antagonist), which also causes seizures in infants who consume breast milk from a mother who has been treated for them.[2] *Ayurveda* explains many drugs that enhance the production of *Stanya* as *Stanya Janana/Stanya Vardhaka.*[3] One such drug is *Vidarikanda* (*Pueraria tuberosa* D.C).[4] *Acharya Dalhana* in *Sushruta Samhita* has described two varieties of *Vidarikanda,*[5] one with the elongated tubers-*Dirghakanda* and the other with tuber resembling the foot of an Elephant-*Hastipadavat*, *Bidali (Dioscorea pentaphylla* Linn.), has the elongated tubers is the variety of *Vidarikanda.*[4,6] *Vidarikanda* (*Pueraria tuberosa* D.C) is listed under the nearly-threatened red listed important medicinal plant species of India,[7] whereas *Bidali* is listed under the least concerned plants, which is available throughout India,[8] is also consumed as both staple food [9] and as medicine to treat many health ailments by the Tribals

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as per the folklore claim.[10] Hence, the galactagogue activity of *Bidali - Dioscorea pentaphylla* Linn. through experimental evaluation was intended to be studied.

Materials and Methods

Collection and Authentication

Bidali Kanda (*Dioscorea pentaphylla* Linn.) fresh samples were collected from Perdur, Udupi &, Badamanavarathekaval, Bengaluru and were authenticated by Dr. Shiva Manjunath M.P, Senior Scientist, Dept. of Dravya Guna at SSCASR, Bangalore.

Preparation of *Kashaya*

The authenticated *Bidali Kanda* (*Dioscorea pentaphylla* Linn) fresh sample was cleaned with water to remove dust and foreign particles, cut into small pieces and was sun dried. Dry sample was checked for any impurities, foreign matter and preserved in air tight container. Dried sample of *Bidali* (*Dioscorea pentaphylla* Linn.) was made into a coarse powder in Laboratory, Department of PG Studies in Dravyaguna, Sri Sri College of Ayurvedic Science and Research, Bengaluru. The obtained coarse powder was collected and stored in zip lock cover and kept inside an air tight container.

Kashaya was prepared daily and administered in fresh form orally. General method of *Kashaya* preparation according to *Acharya Sharangadhara*[11] was followed i.e., 1 part of drug, 8 parts of water reduced to 1/4th (1:8-1/4) and was then filtered using a Khora cloth.

Experimental Study

The experimental procedure was carried out in accordance with the ethical guidelines for animals proposed by CPCSEA, Government of India. The Animal ethics clearance was obtained from Department of Pharmacology, Acharya & B.M Reddy College of Pharmacy, Bengaluru as per the protocol outlined in publication of the Committee for the Purpose of Control and Supervision of Experiments on Animals Standards Guidelines (CPCSEA) and approval was obtained from Institutional Animal Ethics Committee (IAEC) with reference no: IAEC/ABMRCP/2023-24/2

Source of Animals

From Biogen Laboratory Animal Facility, Bengaluru, twenty-four healthy mature female Wistar albino rats required for the study were procured.

Acute Toxicity Study[12]

Acute oral toxicity - Limit test of *Bidali Kanda* (*Dioscorea pentaphylla* Linn.) *Kashaya* was conducted according to OECD guidelines 425. Nulli-parous, non-pregnant, healthy Wistar rats of 10-12 week-old, weighing about 180-200g were selected.

A single dose of *Bidali* (*Dioscorea pentaphylla* Linn.) was given in the dose of 2000mg/Kg p.o., was given and observed for 14 days.

For 14 days, a single dosage of *Bidali* (*Dioscorea pentaphylla* Linn.) at a dose of 5000 mg/Kg p.o. was administered and monitored.

Animals were monitored every 30 minutes for the first 4 hours after dosing, then on occasion for the next 24 hours, and then every day for the next 14 days.

Table 1: The table denotes the observation particulars during the Acute Toxicity Study.

Observed for	
Behavioural Profile	Spontaneous, restlessness, irritability, and fearfulness
Neurological Profile	Spontaneous activity, reactivity, touch response, pain response and gait
Autonomic Profile	Defecation and urination
Other Changes	Change in skin or fur, eyes, mucosa and respiratory, Somatomotor and tremors, convulsion, diarrhoea, lethargy, sleep, coma, changes in the weight and any mortality.

Galactagogue Activity[13]

Housing and feeding

The standard guidelines for the housing of the animals mentioned in the OECD (Organization for Economic Co-operation and Development) 425 Guidelines were followed- Animals were maintained in cages with

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

paddy husk as bedding and housed at an ambient temperature 24 0C and humidity with 12-hours light and 12-hours dark cycles. Animals had free access to food and portable water.

Preparation of animals and Grouping

Randomly selected rats were divided into 4 groups consisting of 6 rats in each group. Each rat was marked for their individual identification as **C** (C1, C2, up to C6), **S** (S1, S2, up to S6), **TL** (TL1, TL2, up to TL6) & **TH** (TH1, TH2, up to TH6). The cages were labeled with the experiment name, dosage group and number of rats in that group. (Figure 5)

Table 2: The table represents the grouping of animals

SN	Group	Drug used	No of rats
1.	Normal control (C)	(normal diet and portable water)	6
2.	Standard control (S)	*Vidari* (*Pueraria tuberosa* D.C-1000mg/kg)	6
3.	Trial drug in Lower Dose (TL)	*Bidali* (*Dioscorea pentaphylla* Linn. - 500mg/Kg)	6
4.	Trial drug in Higher Dose (TH)	*Bidali* (*Dioscorea pentaphylla* Linn. - 1000mg/Kg)	6

Study Protocol

For weight gain of pups

24 lactating dams weighing 200 – 250 g at the beginning of lactation and suckling their pups were used for this experiment. Females were divided into 4 experimental groups and received normal diet and portable water (n=6), the standard group were administered with *Kashaya* of *Pueraria tuberosa* D.C (1000mg/Kg) (n=6) and 2 trial groups were administered with the *Kashaya* of *Dioscorea pentaphylla* Linn. In D1 and D2 doses (D1 and D2 were calculated after Acute Toxicity Study: D1-Lower dose and D2-Higher dose). All animals were treated daily, starting on the evening of day 1 of lactation. The *Kashaya* was administered orally with a gavage syringe each day at 10AM. Milk production was measured from 1 day to day 15 of lactation. Milk yield and body weight of dams, and weight gain of pups were measured each day with an electronic balance. Every day during the study period, the pups were weighed (Figure 8) at 9AM (w1) and subsequently isolated from their mother for 4 h (Figure 7) (Sampson & Jansen 1984). At 12PM, the pups were weighed (w2), and returned to their mother and were allowed to feed for 1 h. At 1PM, they were weighed (w3). Milk yield was estimated as w3 – w2. Daily milk yield was corrected for weight loss due to metabolic processes in the pup (respiration, urination, and defecation) during suckling. The value used was (w2 – w1)/4. This value was then be multiplied by the number of suckling hours per day and added to the daily suckling gain (Sampson & Jansen 1984). Daily weight gain of pups was calculated from the pup weight at w2.

Finally, the milk yield was calculated by the following formula;

Milk yield (g) = (W3-W2) + [(W2-W1)/4]

Where; (W3-W2)-weight gain of pups after lactating/feeding

(W2-W1)/4-weight loss due to metabolic processes in the pups

For Serum Prolactin Concentration

The samples were collected on 1st, 14th and 21st day of the study and were estimated by ELISA method from two rats from each group.

For Histopathology of breast tissue

At the end of study, the breast tissue was dissected under local anaesthesia from three rats from each group.

Parameters of the study

Physical parameters; weight of the dams and weight of the pups. Biochemical parameters; Serum prolactin concentration- was done on 1st, 14th and 21st day on two rats from each group. Histopathological assessment: Histopathological study of breast tissue- Was done at the end of study; the samples were being collected from three rats from each group.

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

Statistical analysis

The collected Data was analysed by repeated measures analysis of variance, Friedman repeated measures analysis of variance and Friedman Repeated measures analysis of variance on ranks followed by Holm Sidak method and Tukey's test. P value < 0.05 was being considered as statistically significant and Sigma Stat 3.1 was used for statistical analysis.

OBSERVATIONS AND RESULT

1. Acute Toxicity Study (Table 3-6)

There were no changes observed either physically or behaviorally from 0th day to 14th day at the dose of 2000mg/Kg p.o. of *Bidali* (*Dioscorea pentaphylla* Linn.). Further, on the dose of 5000mg/Kg p.o., of *Bidali* (*Dioscorea pentaphylla* Linn.) no significant physical and behavioral changes were noted.

Table 3: Changes recorded in animals during Acute Toxicity Study of *Bidali* (*Dioscorea pentaphylla* Linn.) at the dose of 2000mg/Kg p.o., orally on 0th day, 7th, and 14th day.

SN	Signs & Symptoms		0th day				7th day	14th day
			Basal	2h	4h	24h		
1.	General impression		N	Active	Active	N	Active	Active
2.	Increased motor activity		-	-	-	-	-	-
3.	Convulsion	Tonic	-	-	-	-	-	-
		Clonic	-	-	-	-	-	-
4.	Straub's reaction		-	-	-	-	-	-
5.	Muscle spasm		-	-	-	-	-	-
6.	Catatonia		-	-	-	-	-	-
7.	Opisthotonos		-	-	-	-	-	-
8.	Hyperesthesia		-	-	-	-	-	-
9.	Decreased motor activity		-	-	-	-	-	-
10.	Muscle relaxation		-	-	-	-	-	-
11.	Anesthesia		-	-	-	-	-	-
12.	Arching and rolling		-	-	-	-	-	-
13.	Lacrimation		-	-	-	-	-	-
14.	Diarrhea		-	-	-	-	-	-
15.	Writhing		-	-	-	-	-	-
16.	Salivation	Viscid	-	-	-	-	-	-
		Watery	-	-	-	-	-	-
17.	Respiration	Depression	-	-	-	-	-	-
		Failure	-	-	-	-	-	-
18.	Skin color	Blanching	-	-	-	-	-	-
		Cyanosis	-	-	-	-	-	-
		Vasodilatation	-	-	-	-	-	-
19.	Grip strength		N	N	N	N	N	N
20.	Visual placing response		N	N	N	N	N	N
21.	Tail pinch response		N	N	N	N	N	N
22.	Auditory response		N	N	N	N	N	N
23.	Mucus membrane		N	N	N	N	N	N
24.	Piloerection		N	N	N	N	N	N

Table 4: CNS & ANS changes recorded during the acute toxicity study of *Bidali* (*Dioscorea pentaphylla* Linn.) at the dose of 2000mg/Kg p.o. on 0th, 7th and 14th day.

SN	Particulars		0th day				7th day	14th day
			Basal	2h	4h	24h		
1.	Exitus		-	-	-	-	-	-
2.	CNS depression	Hypo activity	-	-	-	-	-	-
		Passivity	-	-	-	-	-	-

ISSN: 2456-3110 **ORIGINAL ARTICLE** January 2024

		Relaxation	-	-	-	-	-	-
		Ataxia	-	-	-	-	-	-
		Narcosis	-	-	-	-	-	-
3.	ANS	Ptosis	-	-	-	-	-	-
		Exophthalmos	-	-	-	-	-	-
4.	CNS stimulation	Hyperactivity	-	-	-	-	-	-
		Irritability	-	-	-	-	-	-
		Stereotypy	-	-	-	-	-	-
		Tremors	-	-	-	-	-	-
		Convulsion	-	-	-	-	-	-
		Straub tail	-	-	-	-	-	-
5.	Others		N	N	N	N	N	N

Table 5: Changes recorded in animals during 0th,7th, 14th day of Acute Toxicity Study of *Bidali* (*Dioscorea pentaphylla* Linn.) at the dose of 5000mg/Kg p.o., orally.

SN	Signs & Symptoms		0th day				7th day	14th day
			Basal	2h	4h	24h		
1.	General impression		N	Active	Active	N	Active	Active
2.	Increased motor activity		-	-	-	-	-	-
3.	Convulsion	Tonic	-	-	-	-	-	-
		Clonic	-	-	-	-	-	-
4.	Straub's reaction		-	-	-	-	-	-
5.	Muscle spasm		-	-	-	-	-	-
6.	Catatonia		-	-	-	-	-	-
7.	Opisthotonos		-	-	-	-	-	-
8.	Hyperesthesia		-	-	-	-	-	-
9.	Decreased motor activity		-	-	-	-	-	-
10.	Muscle relaxation		-	-	-	-	-	-
11.	Anesthesia		-	-	-	-	-	-
12.	Arching and rolling		-	-	-	-	-	-
13.	Lacrimation		-	-	-	-	-	-
14.	Diarrhea		-	-	-	-	-	-
15.	Writhing		-	-	-	-	-	-
16.	Salivation	Viscid	-	-	-	-	-	-
		Watery	-	-	-	-	-	-
17.	Respiration	Depression	-	-	-	-	-	-
		Failure	-	-	-	-	-	-
18.	Skin color	Blanching	-	-	-	-	-	-
		Cyanosis	-	-	-	-	-	-
		Vasodilatation	-	-	-	-	-	-
19.	Grip strength		N	N	N	N	N	N
20.	Visual placing response		N	N	N	N	N	N
21.	Tail pinch response		N	N	N	N	N	N
22.	Auditory response		N	N	N	N	N	N
23.	Mucus membrane		N	N	N	N	N	N
24.	Piloerection		N	N	N	N	N	N

Table 6: CNS & ANS changes recorded during the 0th, 7th, 14th day of acute toxicity study of *Bidali* (*Dioscorea pentaphylla* Linn.) at the dose of 5000mg/Kg p.o.

SN	Particulars		0th day				7th day	14th day
			Basal	2h	4h	24h		
1.	Exitus		-	-	-	-	-	-
2.	CNS depression	Hypo activity	-	-	-	-	-	-
		Passivity	-	-	-	-	-	-
		Relaxation	-	-	-	-	-	-
		Ataxia	-	-	-	-	-	-

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

		Narcosis	-	-	-	-	-	-
3.	ANS	Ptosis	-	-	-	-	-	-
		Exophthalmos	-	-	-	-	-	-
4.	CNS stimulation	Hyperactivity	-	-	-	-	-	-
		Irritability	-	-	-	-	-	-
		Stereotypy	-	-	-	-	-	-
		Tremors	-	-	-	-	-	-
		Convulsion	-	-	-	-	-	-
		Straub tail	-	-	-	-	-	-
5.	Others		N	N	N	N	N	N

Dose fixation:

As there was no toxicity observed at the dose of 5000mg/Kg p.o., the Lower dose was fixed as 500mg/Kg p.o., and Higher dose as 1000mg/Kg p.o..

2. Estimation of Milk Yield by the means of Pup Weight and Weight Gain (Galactagogue activity):

Assessment criteria and results:

i. Weekly average of weight of Dams

Table 7: Table showing the changes in weekly weight of Dams in different groups.

Groups	Control	Standard (*Pueraria tuberosa* D.C-1000mg/kg)	TL (*Dioscorea pentaphylla* Linn. -500mg/kg)	TH (Dioscorea pentaphylla Linn-1000mg/kg)	Significance
Week 1	181.263 ±7.326	180.792 ±8.275	194.667 ±4.763	181.155 ±9.577	NS
Week 2	189.175 ±6.760	188.015 ±8.959	225.517 ±17.693	186.318 ±4.947	NS
Week 3	197.023 ±4.739	198.825 ±9.736	241.682 ±18.003	205.752 ±8.709	NS
Significance	**HS (P <0.001)**	**HS (P <0.001)**	**S (P <0.006)**	**HS (P <0.001)**	

The above table describes the weekly change in the body weight of Dams, expressed as mean ±SEM, (n=6), where, TH-- Trial drug (*Dioscorea pentaphylla* Linn.) in Higher dose (1000mg/Kg p.o.) and TL- Trial drug (*Dioscorea pentaphylla* Linn.) in Lower dose (500mg/Kg p.o.).

- The statistical analysis was done by using one-way repeated measure analysis of variance, **between the groups** there was no Statistical significance seen even though there was apparent difference were observed (NS-Not Significant).
- **Within the groups**, the statistical analysis was done using one-way repeated measures analysis of variance, significant after the analysis of variance followed by Holm-Sidak method, where highly significant difference was seen in Control, Standard and Trial Drug with Higher Dose group with **P<0.001** and Significant difference was observed with **P<0.006.**

ii. Milk Estimation by the means of Pup Weight and Weight gain

a. Milk Estimation by means of Pup weight:

Table 8A: Daily milk production by the means of weight gain of Pups from day 1 to day 7 expressed as mean ±SEM (n=6) (between).

Groups	Daily milk production by the means of weight gain of Pups from day 1 to day 7 (g)						
	D1	D2	D3	D4	D5	D6	D7
Control	0.206 ±0.08	0.0821 ±0.0168	0.105 ±0.0511	0.0392 ±0.00701	0.137 ±0.0294	0.173 ±0.0674	0.116 ±0.00835
Standard (*Pueraria tuberosa*-1000mg/kg)	0.0465 ±0.016	0.0608 ±0.008	0.0770 ±0.015	0.144 ±0.031	0.057 ±0.012	0.186 ±0.061	0.168 ± 0.03

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

TL (*Dioscorea pentaphylla* Linn. - 500mg/kg)	0.161 ±0.06	0.161 ±0.06	0.245 ±0.063	0.183 ±0.05	0.107 ±0.04	0.185 ±0.06	0.224 ±0.13
TH (*Dioscorea pentaphylla* Linn- 1000mg/kg)	0.0675 ±0.028	0.119 ±0.021	0.104 ±0.038	0.0802 ±0.021	0.0673 ±0.021	0.137 ±0.054	0.0830 ±0.022

Table 8B: Table showing daily milk production by the means of weight gain in Pups (g) the from day 8 to day 15 expressed as mean expressed as mean ±SEM (n=6).

Groups	Daily milk production by the means of weight gain in Pups (g) from day 8 to day 15							
	D8	D9	D10	D11	D12	D13	D14	D15
Control	0.0921 ±0.00645	0.185 ±0.0114	0.0839 ±0.00377	0.150 ±0.0353	0.124 ±0.0124	0.127 ±0.0198	0.127 ±0.0181	0.0810 ±0.00733
Standard (*Pueraria tuberosa*- 1000mg/kg)	0.0953 ±0.0270	0.0922 ±0.0250	0.0741 ±0.0205	0.203 ±0.0424	0.130 ±0.0334	0.0935 ±0.0141	0.119 ±0.0247	0.136 ±0.0565
TL (*Dioscorea pentaphylla* Linn. - 500mg/kg)	0.264 ±0.0775	0.191 ±0.0558	0.190 ±0.0504	0.205 ±0.0488	0.107 ±0.0482	0.264 ±0.0826	0.131 ±0.0363	0.393 ±0.132
TH (*Dioscorea pentaphylla* Linn- 1000mg/kg)	0.111 ± 0.0241	0.146 ±0.0341	0.170 ±0.0487	0.231 ±0.0803	0.165 ±0.0674	0.128 ±0.0299	0.169 ±0.0584	0.209 ± 0.0610

- The data related to the daily milk production by the means of weight gain in pups (g) on different days have been depicted in Table 9A & 9B. Comparison of D1 milk production by the means of weight gain in pups (g) was done by the milk production by the means of weight gain in pups (g) recorded on D2 to D15.
- **Between the groups,** no significant difference could be observed in milk production by the means of weight gain in pups (g) as measured on D1 to D15 even though there was apparent increase in the same during the treatment course.
- **Within the group; Control group,** Statistical analysis was made using repeated measures ANOVA, followed by Tukey's test, where statistical significance was found with a **p<0.007.** In **Standard Group,** Statistical analysis was made using repeated measures of ANOVA, where statistical difference was not found. In **TL Group,** Statistical analysis was carried out by using Friedman repeated measures ANOVA, where no statistical difference was noted. In **TH Group:** Statistical analysis was carried out by using Friedman repeated measures of ANOVA, where no statistical difference was noted.

b. Pup Weight Gain

Table 9A: The table describes the data related to daily pup weight gain (g) from day 1 to day 7 expressed in Mean and ±SEM (n=6) (between the group)

Groups	Daily Pup weight gain from day 1 to day 7 (g)						
	D1	D2	D3	D4	D5	D6	D7
Control	6.116 ±0.377	6.209 ±0.472	6.847 ±0.697	7.929 ±0.784	9.151 ±0.980	10.251 ±1.242	11.782 ±1.459
Standard (*Pueraria tuberosa*-	6.390 ±0.124	7.046 ±0.223	7.866 ±0.391	8.789 ±0.567	10.101 ±0.584	11.234 ±0.705	12.524 ±0.845

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

1000mg/kg)							
TL (*Dioscorea pentaphylla* Linn. - 500mg/kg)	6.420 ±0.269	7.383 ±0.351	8.438 ±0.467	9.726 ±0.622	10.912 ±0.807	12.017 ±0.964	13.116 ±1.124
TH (*Dioscorea pentaphylla* Linn. - 1000mg/kg)	6.067 ±0.195	6.349 ±0.212	7.402 ±0.288	8.496 ±0.459	9.526 ±0.462	10.680 ±0.507	12.064 ±0.493

Table 9B: The table describes the data related to daily pup weight gain(g) from day 8 to day 15 expressed in Mean and ±SEM (n=6) (between the group)

Groups	Daily Pup weight gain from day 8 to day 15 (g)							
	D8	D9	D10	D11	D12	D13	D14	D15
Control	13.385 ±1.760	14.951 ±1.957	16.618 ±2.128	17.740 ±2.306	18.830 ±2.553	19.840 ±2.511	21.645 ±3.051	23.502 ±3.457
Standard (*Pueraria tuberosa*-1000mg/kg)	13.962 ±0.853	15.404 ±0.922	16.926 ±1.021	18.329 ±1.015	19.601 ±1.145	20.670 ±1.138	22.911 ±1.516	24.239 ±1.511
TL (*Dioscorea pentaphylla* Linn. - 500mg/kg)	14.074 ±1.300	15.140 ±1.424	16.926 ±1.557	18.109 ±1.683	19.058 ±1.797	20.873 ±1.894	22.276 ±2.001	23.6 ±2.162
TH (*Dioscorea pentaphylla* Linn. - 1000mg/kg)	13.275 ±0.499	14.659 ±0.498	15.490 ±0.360	16.867 ±0.309	18.347 ±0.240	20.086 ±0.285	21.359 ±0.325	22.815 ±0.547

- The above table 15A &B depicts the daily weight gain of Pups (g) from day 8 to day 15 daily weight of Pups (g), expressed in Mean and ±SEM (n=6). where TH- Trial drug (*Dioscorea pentaphylla* Linn.) in Higher dose (1000mg/Kg p.o.) and TL- Trial drug (*Dioscorea pentaphylla* Linn.) in Lower dose (500mg/Kg p.o.)
- **Between the Group;** the statistical analysis was carried out using repeated measures of one-way analysis of variance, **where there was no statistical significance was elucidated.**
- **Within the group; In Control Group:** Statistical analysis carried out by using Freidman repeated measures one way analysis of variance on rank, followed by Tukey Test; where the results are **Highly significant; p<0.001** from Day 1 to Day 15. In **Standard Group:** Statistical analysis carried out by using repeated measures of one-way analysis of variance, followed by holm Sidak method: where the results are **highly significant; p<0.001** from Day 1 to Day 15. In **TL Group**: Statistical analysis carried out by using repeated measures of one-way analysis of variance, followed by Tukey Test: where the results are **highly significant; p<0.001** from day 1 to day 15. In **TH Group:** Statistical analysis carried out by using repeated measures of one-way analysis of variance, followed by Holm Sidak method: where the results are **highly significant; p<0.001.**

iii. Serum prolactin concentration

Table 10: Table showing the serum prolactin concentration of different groups.

SN	Serum prolactin concentration(ng/dl)				
	Groups		Day1	Day14	Day 21
1.	Control	C1	<0.47	1.29	<0.47
		C2	<0.47	<0.47	<0.47
2.	Standard (*Pueraria tuberosa*-1000mg/kg)	S1	<0.47	<0.47	<0.47
		S2	<0.47	<0.47	<0.47
3.	TL	TL1	<0.47	<0.47	<0.47

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

	(*Dioscorea pentaphylla* Linn. - 500mg/kg)	TL2	0.68	<0.47	<0.47
4.	TH (*Dioscorea pentaphylla* Linn- 1000mg/kg)	TH1	<0.47	<0.47	<0.47
		TH2	4	<0.47	<0.47

iv. Histology of Breast Tissue

Table 11: The table showing findings of Histology of Breast Tissue in all four groups.

Figure 1: Control group

Section studied shows increase in tubule-alveolar unit with focal distorted alveoli [Fig 1A, arrow] and devoid of secretions. At focal areas hyperplasia of alveoli with distinct luminal lined by cuboidal to columnar epithelium, few containing intra-luminal secretions [Fig.1B, Arrow] and surrounded by basement membrane are seen. The intervening fibro-collagenous stroma appears variably.

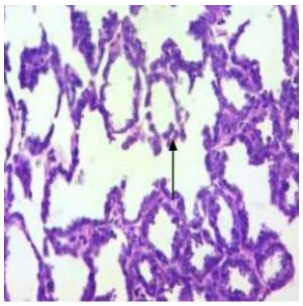

Figure 1A

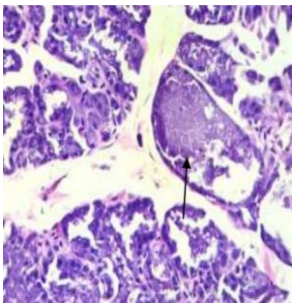

Figure 1B

Figure 2: Standard Group

Section studied shows increase in tubulo-alveolar unit with hyperplasia of alveoli in the hypodermis. The alveoli have distinct lumina lined by columnar epithelium with intraluminal secretions and surrounded by basement membrane [Fig.2A, Arrow]. The epithelium appears hypertrophied at some places. The intervening fibro-collagenous stroma appears reduced [Fig.2B, Arrow]

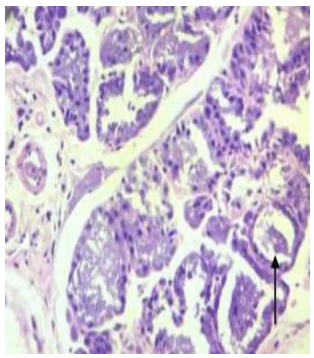

Figure 2A

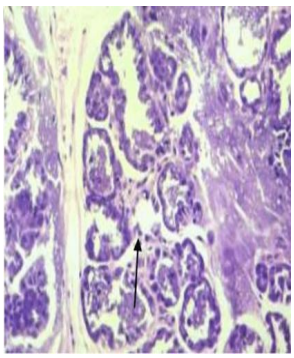

Figure 2B

Figure 3: TL Group

Section studies showed decreased Tubulo- alveolar unit with alveoli in the hypodermis. The alveoli have distinct lumina lined by single layer of cuboidal epithelium surrounded by thick basement membrane [Fig.3A, Arrow] and interstitium. The alveoli are spaced with intervening dense fibro collagenous stroma [Fig.3B, Arrow].

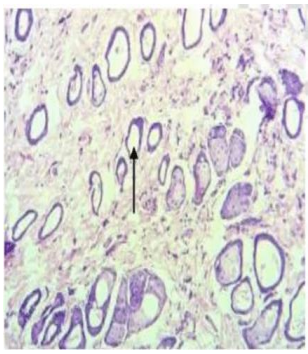

Figure 3A

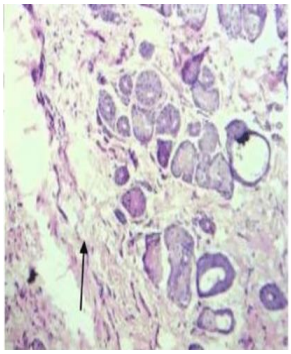

Figure 3B

Figure 4: TH Group

The section studied showed increase in tubule-alveolar unit with hyperplasia of alveoli in the hypodermis. The alveoli have distinct lumina lined by columnar epithelium with intra-luminal secretions and surrounded by basement membrane [Fig.4A, Arrow]. The epithelium appears hypertrophied and hyperplastic at some places. The intervening fibro-collagenous stroma appears reduced [Fig.4B, Arrow].

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

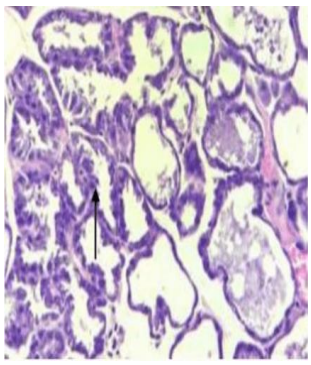

Figure 4A

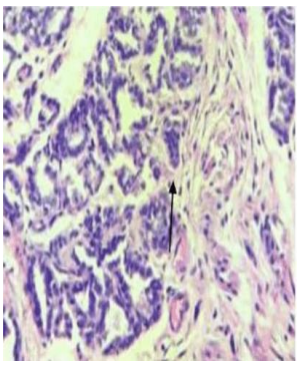

Figure 4B

DISCUSSION

Acharya Dalhana in *Nibandhasangraha* has described two varieties of *Vidarikanda* while commenting on the same in *Sushruta Samhita*, Viz., One with tubers that resemble the foot of an Elephant- *Hastipadavat*, and the other with tubers that are elongated- *Dirghakanda*.[4]

Bidali (*Dioscorea pentaphylla* Linn.), which is abundantly found throughout India, has the elongated tubers-*Dirghakanda*, is well accepted by K C Chunekar & Thakur Balwant Singh as the variety of *Vidarikanda*.[4,6] It also is used as the vegetable/staple food in the Western Ghats and North-Eastern parts of India.[9] Hence, *Bidali* was chosen for study its Galactagogue action by experimental evaluation.

Weight of Dams

Earlier, in the study related to assessment of milk production, the breast of the dams under the study were dissected and weighed to infer the increase in milk production, Later the procedure was modified, and the body weight of dams (as whole) was considered to infer the same.[14]

In the present study, increase in body weight of dams were noted in all the four groups, although there was no statistical significance between the groups, Statistical significance was observed within the groups (Control- P <0.001, Standard- P <0.001, Trial Drug-*Dioscorea pentaphylla* Linn. in lower dose: 500mg/Kg- P <0.006 and Trial Drug-*Dioscorea pentaphylla* Linn. In higher dose:1000mg/Kg-<0.001).

Milk Estimation by the means of Pup Weight and Weight gain

a. Milk Estimation by means of Pup weight:

Weight increase in the litter/ pups is frequently employed as a measure of milk production since it provides an estimate of milk productivity which is corrected by the weight loss due to metabolic activities like excretion and respiration in pups.[13,14] Although there was increased milk estimation by the means of weight but was not up to the statistical significance in all the groups.

b. Pup weight Gain

The Pups/ Litter growth is an indicative of adequate milk production by their dams for pup nourishment.[13,14] Here was statistical significance within the group with P <0.001 in all the four groups, but between the groups there was no statistical significance.

Prolactin

The initiation of lactation, the composition of milk's macronutrients, and milk production all depend on the main lactogenic hormone, Prolactin, which is secreted by the anterior pituitary. Its levels in the blood rise throughout pregnancy to a level that is 10 to 20 times higher than usual at the end of gestation.[15]

Rat prolactin (rPRL), a single-chain polypeptide hormone produced by the rat anterior pituitary, has a mass of about 23,000 molecules. The amino acid sequence of prolactin varies significantly between species. About half of all residues in rat prolactin are different from those in human prolactin.[16]

In an experiment, Rats were used to evaluate serum prolactin levels, and the results revealed that the serum prolactin level increased on the first postpartum day and persisted high for at least 8 days due to the suckling stimulus. Serum prolactin levels decreased to about half of what they were on the first post-partum day on the 15th and 23rd days after delivery. When litters were removed from mother rats on the fourth postpartum day, serum prolactin dropped quickly and returned to pregnancy levels three hours later.[17]

In the current study, the serum prolactin levels were <0.47 ng/dl, except on 14th day in Control group

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

(1.29ng/dl), 1st day of TH group (*Dioscorea pentaphylla* Linn-1000mg/Kg) (4ng/dl), and 1st day of TL group (*Dioscorea pentaphylla* Linn. -500mg/Kg) (0.68ng/dl).

The result- (uniform, unchanged, undetectable) might be due to lack of suckling reflex (nipple stimulation), which is also a cause for the secretion of Prolactin [18] or due to the insensitivity of the ELISA kit used for the estimation (below the detection level).

Histology of Breast tissue

Parenchyma and interstitial tissue were seen in the normal breast tissue samples taken from non-inbred adult rats. The terminal ducts and acini that made up the parenchyma were homologous to the terminal duct-lobule in the human breast. Fatty tissues made up most of the interstitial tissues. Ductal epithelial cells, myoepithelial cells, and basement membrane made up the three layers of normal ducts. The ductal epithelial cells were constituted of one layer of slender, club-shaped cells made up of two types of cells: dark cells and intermediate cells. A single layer of cells, mostly dark cells with sporadic myoepithelial cells, lined the acini.[19]

In the present study, the animal receiving the *Bidali* (*Dioscorea pentaphylla* Linn.) in higher dose (1000mg/Kg) breast tissue histology revealed increased tubule-alveolar unit with hyperplasia of alveoli in the hypodermis, The alveoli have distinct lumina lined by columnar epithelium with intra-luminal secretions and surrounded by basement membrane and in the group receiving the lower dose (500mg/Kg) of trial drug *Bidali* (*Dioscorea pentaphylla* Linn.) breast tissue showed very few tubulo-alveolar unit with fewer alveoli in the hypodermis and thick basement membrane. This is suggestive of that the *Bidali* (Dioscorea pentaphylla Linn.) in the higher dose (1000mg/kg), promoted the growth of tubule-alveolar unit, and intra-luminal secretion.

Mode of action of *Bidali* on *Stanya Janana*

Mode of action

Shleshma-Vardhaka Dravya Prayoga is advocated in *Stanya Janana*, due to the *Samanya Guna* between *Shleshma* and *Stanya*[20]

Table 12: The Table showing the Mode of Action of *Bidali* through *Rasapanchaka* and its *Karma*

Rasapanchaka		***Karma***
Rasa	*Madhura*	*Dhatu Bala Pradhana* *Stanya-Shleshma Vardhana* *Brihmana*[21]
Guna	*Guru*	*Shleshma Vardhana* *Bala Vardhana* *Brihmana*[22]
	Shita	*Shleshma Vardhana*[22]
	Snigdha	*Shleshma Vardhana*[22]
Virya	*Shita*	*Shleshma Vardhana*[23]
Vipaka	*Madhura*	*Shleshma Vardhana*[24]
Karma		*Rasayana,* *Brihmana*[25] *Stnaya Vardhana* [26]

Therefore, because of the above *Guna-Karma*, its advocation in *Stanya Kshaya* is well justified where it counters the *Samprapti* of *Stanya Kshaya* and increases the *Stanya* to adequate quantity.

Mode of action based on Phytochemical constituents:

- Qualitative Phytochemical analysis showed the presence of Alkaloids, Flavonoids, Tannins, Saponins, Steroids, Carbohydrates, Phenols. On quantification of Alkaloids, Tannins, Flavones, and Saponins, Flavones was found in more quantity among them.[27]
- Diosgenin (steroidal sapogenin), A phytoestrogen which exerts agonist effect and effects on the anterior pituitary to produce Prolactin secretion in Lactotrophs.[28]

ISSN: 2456-3110 ORIGINAL ARTICLE January 2024

- Flavonoid, is known to have effect on ERα (Alpha Estrogen Receptor), which promotes growth of Mammary gland and Production of Prolactin Hormone.[29]
- The alkaloids in the drug function as a protein synthesis reservoir and accelerate metabolism, which may also explain why both Dams and Pups have gained weight.[30]
- The presence of steroids in the drug aids in the stimulation of growth factor and its impact on bones, muscles, and adipose tissue, which may have contributed to weight gain in dams and pups as well as the development of the mammary gland.[30]
- The influence of steroids on the growth of adipose tissue aids in the production of prolactin hormone since adipose tissue also produces prolactin hormone.[18,30]

CONCLUSION

Bidali (*Dioscorea pentaphylla* Linn.) is one among the two varieties of *Vidari*. The model estimation of milk yield by the means of pup weight and weight gain on Wistar albino rats was done. Outcome of the present study showed better results in the group treated with the Trial drug in dose dependent manner, *Bidali* (*Dioscorea pentaphylla* Linn.) in terms of Dam weight, Daily Milk Yield in means of pup weight and pup weight gain and Histology. The statistical comparison revealed that the difference was insufficient to reject the null hypothesis. Thus, based on this experimental study, it can be concluded that *Bidali* acts as both *Stanya Janana* and *Stanya Vradhaka*.

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http://dx.doi.org/10.21760/jaims.9.1.4

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Figure 5: Grouping and housing of Animals

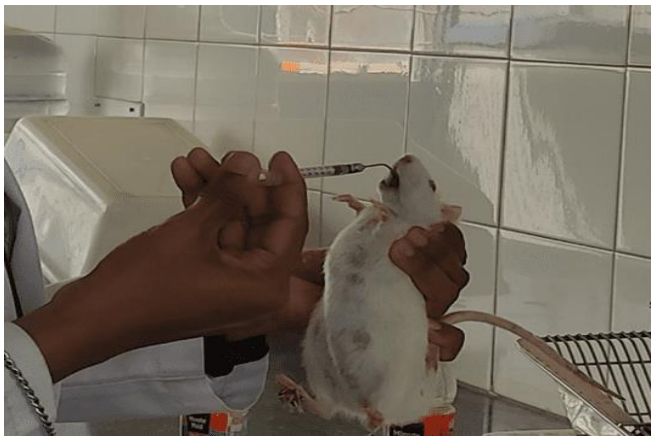

Figure 6: Dosing of Animal

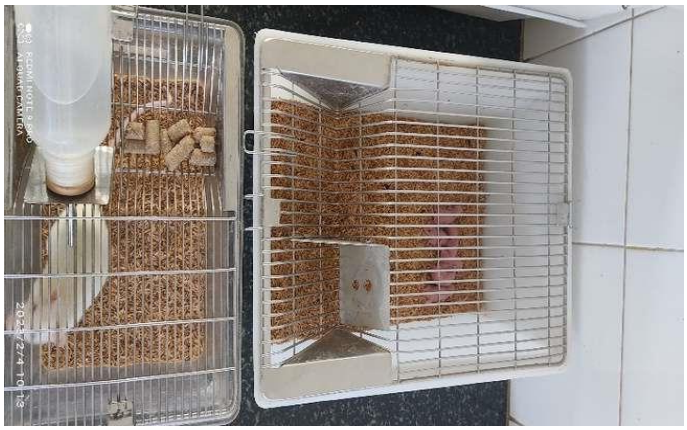

Figure 7: Separation of Dams and Pups

Figure 8: Weighing of Pup

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