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EFFECT OF DARU-SARSHAP-MUSTA LEPA ON WEIGHT OF INTERNAL ORGAN OF ALBINO MICE DUE TO BHALLATAKA INDUCED TOXICITY. (EXPERIMENTAL STUDY)

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ABSTRACT

Bhallataka (*Semecarpus anacardium Linn*.) is a potent drug having high priority and applicability in Ayurveda and has indication for many ailments. In contrast to the critical analytical approach of contemporary medicine, holistic treatment is the hallmark of Ayurvedic treatment. All these facts demand for the research to find out safe, cost effective and efficient Ayurvedic formulation to treat the *Bhallataka* induced dermal toxicity and observe its effects on internal organs Hence, the current study is aimed to develop a new standard herbal formulation with its in-vivo study to screen its effectiveness in swiss albino mice. The obtain results and conspicuous observation have been discuss with scientific reasoning. Effect of antidote lepa was previously done by researcher, its further step to observed its effects on internal organs of albino mice.

KEYWORDS: *Bhallataka* toxicity, *daru-sarshap-musta lepa*, weight of internal organs of albino mice.

INTRODUCTION

Bhallataka is mentioned under *Upavisha* group in Ayurvedic classics like *Rasataranagini*.[1] It is also mentioned as a poisonous medicinal plant in Drugs and Cosmetics Act (India), 1940 *Bhallataka* has potent local action. In Ayurveda *Bhallataka* has great therapeutic value in *agnikarma* for treating pain. Pharmacological action of medicine and poison depends upon *guna, virya, veepaka* of the substance.[2] All the parts of *Bhallataka* are poisonous.[1][2] Tarry

oil present in the pericarp of the fruit causes blisters on contact. There are many documented cases of accidental topical poisoning caused by *Bhallataka*.[3] Various medicinal preparations contain *Bhallataka* as their principal component where it is applied locally as well as consumed internally after *shodhana*.[4] In spite of such major reputations many physicians avoid *Bhallataka* based preparations due to fear of its toxic nature and pharmaceutical units prefer keeping away making its formulations. It becomes important to fine-tune the margins of the safety and adverse effects of toxic plants used in Ayurveda medicine.

Many poisons are used in Ayurveda only after bio purification methods. Still this life science gives remedies to combat their toxic effects if occurs.

Several formulations are given in classics to combat the local action of this *Upvisha*. They act as antidotes. '*Daru-Sarshap-Musta lepa'* for local effects of *Bhallataka* is mentioned in *Rasjalnidhi* and *Anupan manjiri*.[5][6] Though the guiding correspondence has been stated, the mechanism behind this antidote action has not been described; neither the possible mode of action has been explored earlier., hence the action of *Daru-Sarshap-Musta lepa'* with *navneet* needs to be validated scientifically. An attempt will be made to add a well-documented scientific study on poison and validate remedial solutions on their toxicity, described in Ayurveda Classics.

MATERIALS AND METHODS

STUDY DESIGN

Experimental study,

This study will be carried out in two parts

1) Standard preparation of drug

2) Preclinical antidote effect of poison in animal model.

1) Standard preparation of drug

1) Crude Material

Bhallataka fruits, 500 gms.

1) *Devdaru 100 gm.*
2) *Sarshap 100 gm.*
3) Musta 100 gm.
4) *Navneet* produced from cow's milk, 100 ml.

MATERIALS

Following raw drugs will be used for research work with the standard references mentioned in *Rasjalnidhi* and *Anupan manjiri*.

Table no. 1: Materials of Lepa.

Sr.no.	Dravya	Family	Latin name	Prayojyang
1	*Bhallataka*	Anacardaceae	*Semecarpus anacardium Linn.*	Fruit
2	*Devdaru*	Pinaceae	*Cedrus deodara Roxb.*	kandsar
3	*Musta*	Cyperaceae	*Cyperus rotundus Linn.*	Tuber
4.	*Sarshap*	Cruciferae	*Brassica campestris Linn.*	Seed
5	*Navneeta*	-	-	-

Collection of materials

Collection of new drugs will be carried out with the help of *dravyaguna* department of institute and Ayurvedic pharmacopeia, INDIA.

METHODOLOGY

- Identification and authentication of drugs
- Preparation of *Bhallataka* oil extract as per sop
- Preparation of *Daru-Sarshap-Musta lepa* as per sop

Method of preparation of *daru-sarshap-musta lepa*

1. Identification, collection and standardization of raw drugs i.e. *devdaru*, *sarshap*, *musta* done.
2. Made fine powder(85-120 mesh)of raw materials in equal amount each content to mixed with each other.
3. Standardization and authentication of Lepa done with pharmacognosy department.
4. Added *navneet* in the required amount to maintain the consistency of lepa. (the quantity of each ingredients was taken as per the guideline of animal ethical committee)

Preclinical antidote effect of poision in animal model

- Study was done in albino mice as per O.E.C.D. guidelines. (Guideline no.402) IAEC permission was taken from respective institute prior to animal study.
- Permission of (**C.P.C.S.E.A.)** committee for the purpose of control and supervision on animal of (**I.A.E.C.**) internal animal ethics committee was taken.

- Only those albino mice will be selected for experiment, which are showing signs of action of *Bhallataka (semicarpus anacardium Linn.).*
- Test drug:

Test Article : *Daru-sarshap-musta Lepa*
Physical State : Semisolid
Quantity : 100 gm
Colour : Pale Brown

- **DOSE SELECTION AND PREPARATION**

***Bhallataka*:** To cover the area of application, respective amount of *Bhallataka* oil was applied to animals.

***Daru-sarshap-musta Lepa*:** The amount sufficient to cover the area of application was applied to animals.

- **Administration Of Test Article**

Test articles were applied topically.

5) IDENTIFICATION

By unique identification number marked by writing on cage tag and by corresponding colour body markings.

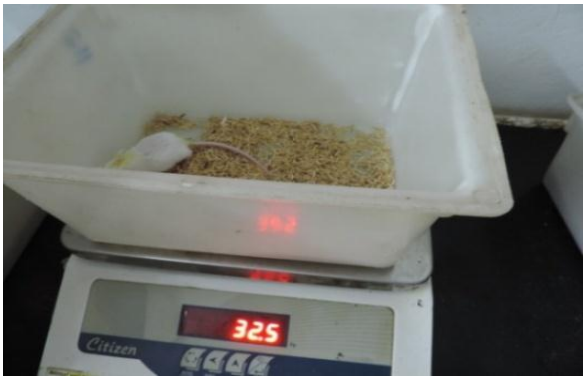
Mice were coded as follows

Code name-meaning.
H: Head
B: Back
T: Tail
HB: Head Back
BT: Back Tail
W: White (No marking)
RF: Right Front Leg
RH: Right Hind Leg
RLS: Right Leg Side
LF: Left Front Leg
LH: Left Hind Leg

LLS: Left Leg Side

Table no. 2: Test System and Management.

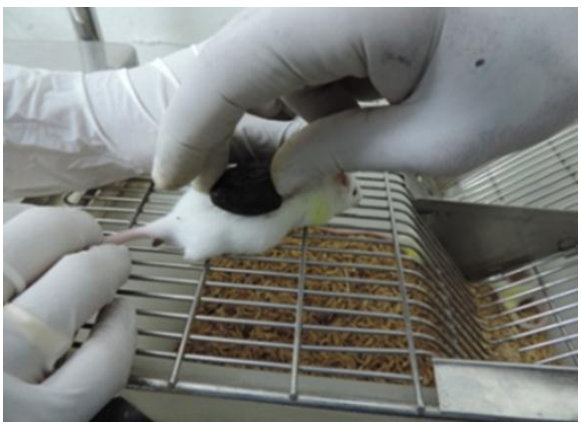
1. Species	:	Mice
2. Strain	:	Swiss Albino
3. Source	:	APT Testing and Research Pvt Ltd, Pune
4. Sex	:	Both male and female
5. Body weight range	:	18.0 g to 20.0 g
6. Identification	:	By unique identification number marked by writing on cage tag and by corresponding colour body markings.
7. No. of animals	:	18
8. Acclimatization	:	The mice were housed in their cages for five days prior to start of dosing in the experimental room after veterinary examination.
Husbandry		
9. Environmental conditions	:	Room temperature maintained between 22±3°C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark.
10. Accommodation	:	Three mice per cage housed in polypropylene cages with stainless steel grill top, facilities for food and water bottle, and bedding of clean paddy husk.
11. Diet	:	Pelleted feed supplied by Supplier.
12. Water	:	Potable water passed through 'Aquaguard' water filter was provided ad libitum in plastic bottles with stainless steel sipper tubes.



Measurement of weight of animal

Trimming of Animal

Marking of animals



Application of Bhallatka

Application of Daru-sarshap-musta lepa

Standard drug Silver nitrate

STUDY DESIGN

Inclusion criterion

Mice were divided into following groups containing 6 animals (3 males and 3 females) per group.

Table no. 3: Inclusion criterion.

Group no.	Group name	Specification (n=6)
1.	DC	Disease Control (*Bhallataka* application)
2.	STD	*Bhallataka* application + STD Drug – Silver nitrate gel (0.2%)
3.	TEST	*Bhallataka* application + *Daru-sarshap-musta Lepa*

Exclusive criteria

Mice does not show any sign after application of *Bhallataka* oil.

Mice shows sign all over the body after application of *Bhallataka* oil.

Observations was made based on Erythema score according to OECD Guidelines 402.

Irritation was scored as follows depending on area and intensity of erythema.

Grading of skin reactions – Acute dermal irritation. (Draize score)

Table no. 4: Gradation on skin.

Test Reaction	Grading Scale
No visible change	0
Discrete Erythema	1
Moderate Erythema	2
Intense Erythema	3
Eschar	4

PROCEDURE

- Every animal of the study group would be marked for identification
- Each animal would be weighted with digital weighing machine.
- Back of each animal was shaven with the help of trimmer and 1 cm×1 cm area marked with the help of picric acid.
- 2-3 *Bhallataka* fruits were punctured with the help of a large needle and holes were made and dilated.
- Uniformly cotton coated plastic stick (ear bud) dipped in to each hole and soaked with the *Bhallataka* oil.
- This method is not a standard method to extract *Bhallataka* oil, however required quantity is very small and in crude form hence this method was adopted.
- Except normal control animals, each animal was applied with approximately 100 µl of Bhallataka oil-soaked swab over pre marked area thoroughly.
- After application procedure, all mice were observed for 24 hours for any signs of toxicity.
- Signs were observed and observations were noted carefully.
- After complete formation of local signs (after 2 hrs) first dose of *Daru-sarshap-musta lepa* (Freshly prepared) was applied to each mouse.
- *Daru-sarshap-musta lepa* was applied twice a day in experimental group with the help of cotton swab, over shaven area, once a day (morning 10 a.m.) for the period of seven days.
- A test sample was applied for 7 consecutive days.
- STD group animals received Silver nitrate gel (0.2%) application.
- All animals were observed daily for 7 days.
- After 7 days animals were sacrificed, and samples were studied for histopathology.

Table no. 5: Inter group comparison of organ weight.

Groups		N	Mean	Std. Deviation	Median	Minimum	Maximum	Chi square value	p value of Kruskal-Wallis Test
Adrenals	1	6	.011333	.0026583	0.0115	.0070	.0140	4.298	0.117#
	2	6	.013833	.0033714	0.013	.0110	.0200		
	3	6	.015000	.0028284	0.0145	.0120	.0200		
	Total	18	.013389	.0032018		.0070	.0200		
Heart	1	6	.270333	.0720324	0.259	.1950	.4010	0.408	0.816#
	2	6	.264333	.0276381	0.264	.2310	.3010		
	3	6	.274833	.0510075	0.2735	.2150	.3410		
	Total	18	.269833	.0503543		.1950	.4010		

Kidneys	1	6	.617833	.1439395	0.628	.4490	.7780	0.46	0.795#
	2	6	.590000	.1699882	0.516	.4460	.8850		
	3	6	.588667	.1191682	0.556	.4410	.7420		
	Total	18	.598833	.1376980		.4410	.8850		
Liver	1	6	2.236167	.5082312	2.424	1.5330	2.7210	0.785	0.675#
	2	6	2.533667	.1536003	2.566	2.3260	2.7120		
	3	6	2.526167	.1394481	2.523	2.3150	2.7010		
	Total	18	2.432000	.3300636		1.5330	2.7210		
Spleen	1	6	.196167	.0509840	0.182	.1450	.2760	5.558	0.062#
	2	6	.163333	.0338625	0.1665	.1120	.2010		
	3	6	.135833	.0400870	0.1235	.1030	.2130		
	Total	18	.165111	.0471005		.1030	.2760		
Lungs	1	6	.270500	.0424771	0.2755	.2020	.3120	2.517	0.284#
	2	6	.261833	.0395141	0.26	.2010	.3150		
	3	6	.234167	.0294918	0.247	.1900	.2630		
	Total	18	.255500	.0387287		.1900	.3150		
Gonads	1	6	.146500	.1150978	0.1465	.0320	.2690	1.133	0.568#
	2	6	.142167	.1194946	0.1405	.0210	.2700		
	3	6	.126333	.1041147	0.1215	.0210	.2380		
	Total	18	.138333	.1066010		.0210	.2700		
Brain	1	6	.357500	.0711667	0.363	.2490	.4590	0.582	0.747#
	2	6	.338000	.0661755	0.3365	.2430	.4500		
	3	6	.350167	.0703659	0.3405	.2510	.4510		
	Total	18	.348556	.0655925		.2430	.4590		
Pancreas	1	6	.144500	.0289603	0.1335	.1190	.1870	0.495	0.781#
	2	6	.146667	.0195721	0.146	.1250	.1810		
	3	6	.141000	.0259538	0.137	.1130	.1850		
	Total	18	.144056	.0237325		.1130	.1870		

There was a statistically non-significant difference seen for the values between the groups ($p>0.05$) i.e. there was no difference between the groups for organ weight.

Table no. 6: Pair wise comparison using Mann-Whitney Test.

Day	Group	Vs group	Mann-Whitney U value	Z value	p value of MW U test
Adrenals	1	2	11	-1.133	0.257#
	1	3	5.5	-2.019	0.043#
	2	3	12.000	-0.973	0.331#
Heart	1	2	14.5	-0.561	0.575#
	1	3	15	-0.48	0.631#
	2	3	16.000	-0.321	0.748#
Kidneys	1	2	14.5	-0.561	0.575#
	1	3	15	-0.48	0.631#
	2	3	15.500	-0.401	0.688#
Liver	1	2	13	-0.803	0.422#
	1	3	14	-0.641	0.522#
	2	3	16.000	-0.320	0.749#

Spleen	1	2	11	-1.121	0.262#
	1	3	4.00	-2.242	0.025*
	2	3	9.500	-1.363	0.173#
Lungs	1	2	15.5	-0.401	0.688#
	1	3	9.00	-1.441	0.15#
	2	3	10.500	-1.203	0.229#
Gonads	1	2	16	-0.32	0.749#
	1	3	11.00	-1.121	0.262#
	2	3	14.500	-0.561	0.575#
Brain	1	2	12.5	-0.882	0.378#
	1	3	17.00	-0.16	0.873#
	2	3	16.000	-0.320	0.749#
Pancreas	1	2	14	-0.641	0.522#
	1	3	18.00	0.000	1.000#
	2	3	14.500	-0.561	0.575#

There was a statistically non-significant difference seen for the values between the groups ($p>0.05$).

Except for spleen wt between group 1 vs 3, there was a statistically significant / highly significant difference seen for the values between the groups ($p<0.01, 0.05$) with higher wt in group 1 as compared to group 3.

DISCUSSION AND RESULTS

The current study was aimed to find out effectiveness of antidote against Bhallataka induced local toxicity on internal organs of albino mice. In the current chapter, the observations and results obtained during the study has been discussed with scientific reasoning.

The experiment protocol was approved by Animal ethical committee in accordance with guidelines formulated by the committee for the purpose of control and supervision of experiments of animals.

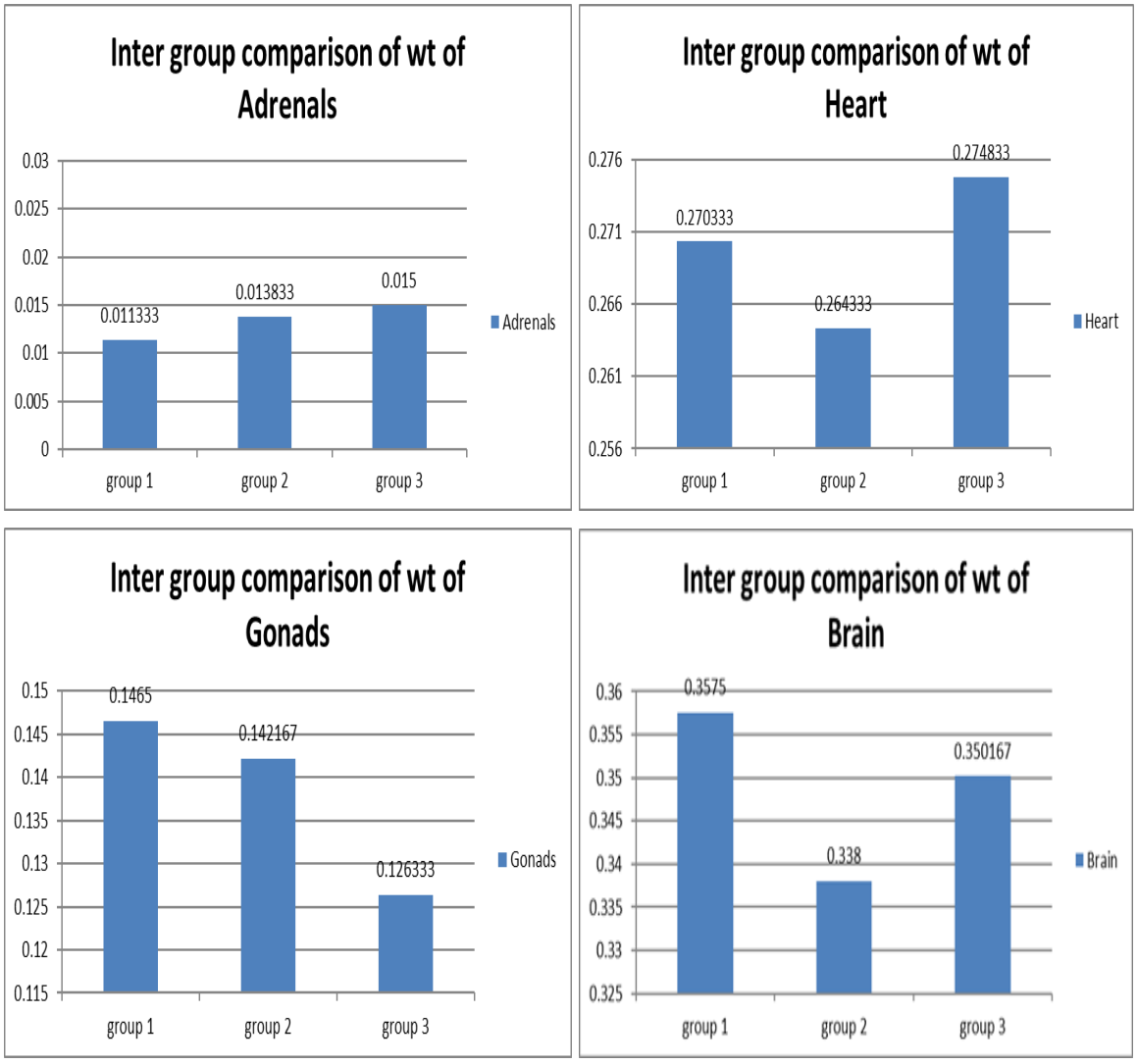
As the anatomical and physiological systems resembles human systems both being mammal, hence the use of swiss albino mice species were selected.

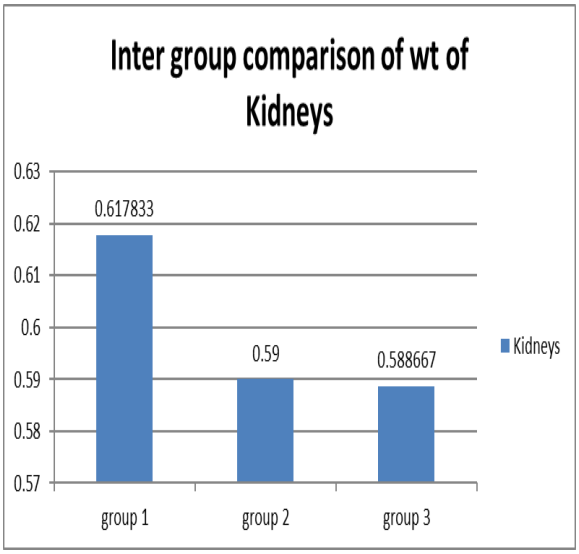
Multiple animals per group were used for statistical reasons in the calculation of result. Considering guidelines for animal model 6 animals were included per group. Experiments Was performed and effects of *Bhallataka* locally was observed. Swelling and Rating of skin reaction was evaluated as per the Indian standards BIS (Bureau of Indian standards) 1992.

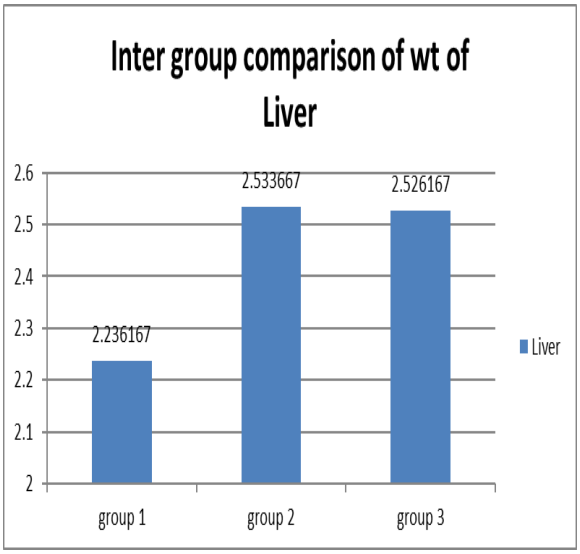
There were changes in weight of organs. Inter group comparison showed changes in weight of different organs. Considering statistical data it was observed that there is no significant changes in weight of internal organs due to effects of antidote *daru-sarshap-musta lepa on Bhallataka* induced toxicity.

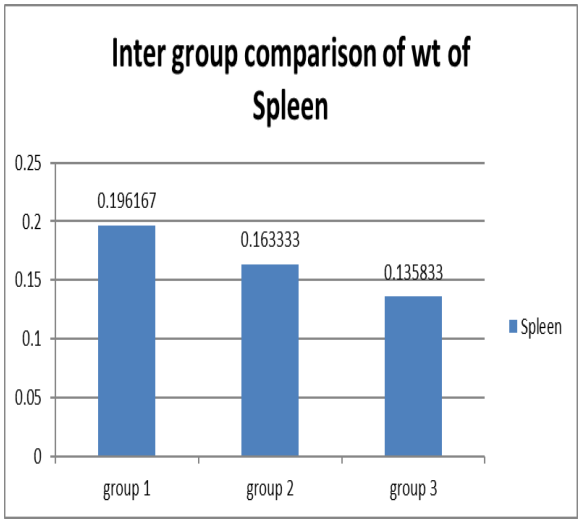
According to contemporary view, *Devdaru* possessed healing[7] as well as antibacterial[8][9] property, *sarshap* has antioxidant nature[10] whereas *musta* possessed anti-inflammatory[11], anti-pyretic and analgesic activities[12], wound healing activity[13], cytotoxic and apoptotic activities.

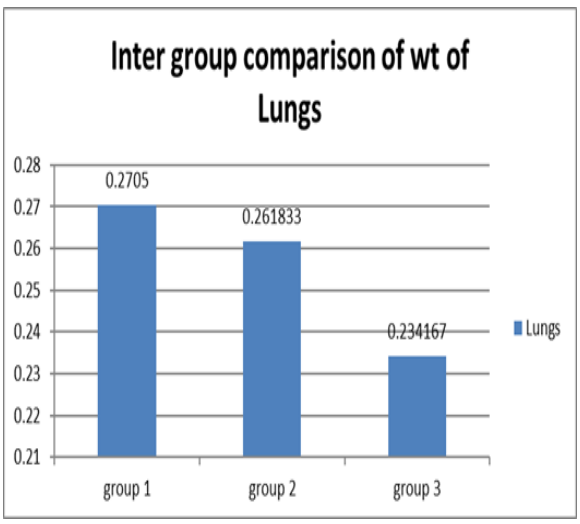
According to histopathological report Antidote lepa showed significant changes in skin manifestation in albino mice but not on internal organs. Therefore, it can be stated that there is least effect of lepa to prevent internal toxicity caused by Bhallataka considering changes in weight of organs.

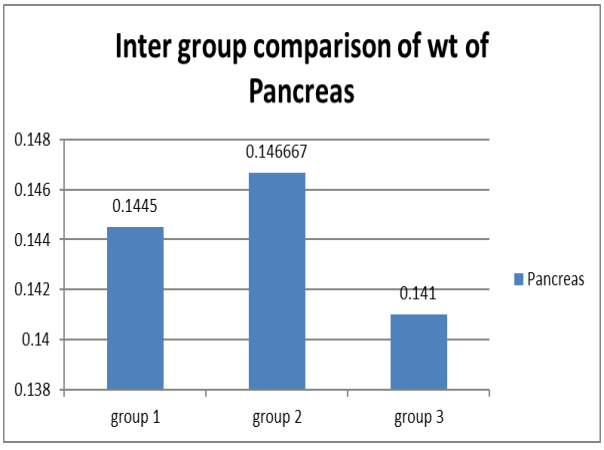












CONCLUSION

In scientific study, observations obtained, results and logical discussions are the key points towards the definite conclusion. In the present study sincere attempts has been made to draw definite conclusion regarding efficiency of *Daru-Sarshap-Musta lepa* on weight of internal organs of albino mice due to *Bhallataka* induced toxicity.

Observation, Statistical data and histopathological reports showed that there is no significant effect of *daru-sarshap-musta lepa* on weight of internal organs except spleen in albino mice due to *Bhallataka* induced toxicity.

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