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In vivo study of Apathya in Kupilubeej poisoning in Albino mice

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ABSTRACT -Agadtantra is the branch of Ayurved where the description about plant, animal and mineral poison is elaborated. These poisons or Vishaare Sthavar, Jangam,Krutrim,Akrutrim,Gara and Dushi. Its effects, symptoms and treatment is well described in Samhitas. In Ayurved,Pathya-Apathya is recommended in the treatment of various diseases. Which are the rules or dos and don'ts in Aahar -Vihar in the regular daily life.In Rasjalnidhi book, ApathyainVishchikitsa is also recommended. This article is on the research done as experimental animal study where Apathyawas given to intoxicated Albino mice. Various parameters were considered for the study. The study showed that an Apathya increased the symptoms of toxicity. Thus proving that the Apathya should be followed in the treatment of toxicity for better recovery.

Key words- Acute oral toxicity, Pathya- apathya, Kupilu,Jambir, Strychnosnux vomica.

I. INTRODUCTION-

FromAshtangaAyurved, Agadtantrais one of the branch which elaborates about poisons which are plant origin,animal origin and mineral origin. Vishautapatti, prakar,adhishthan, symptoms of toxicity,it's treatment is well described in CharakSamahita,SushrutSamhita,Ashtanghruday,Bhavprakash,Rastaringinietc.In Ayurved, Pathya - Apathya is recommended in the treatment of various diseases or in any Avastha(stage)of many diseases for the better recovery. In Rasjalnidhi book, Apathya is recommended to thetoxicated person with the conventional treatmentof poison.[1]It warns indirectly that if we do not follow this, the toxic effects of any poisonous drug will increase. So, rationale is that, we can advise to follow these rules to toxicated person, so it will help the person to recover from effects of toxicity. And thus it will be beneficial for the community as well.

In this experimental animal research study, one of theApathyarecommended was given to the Albino mice,in which already acute toxicity was produced by giving orally a plant poison.

Aim: To study the effect of Apathya in Kupilu beej poisoning.

Objectives:

1) To find the LD50 dose.
2) To study the toxic effect of Kupilu beej.
3)To study the effect of Apathya i.e. Amla rasin Kupilu beej poisoning.

II. REVIEW OF LITERATURE

Introduction of Kupilu (strychnos nux vomica)

Kupilu description in Samhitas

Kupilu (Strychnos nux-vomica L.), is a well-known plant in Ayurveda since long time and is being used extensively nowadays. There are a number of classical formulations in Ayurved with great therapeutic importance. TheKupilu plant is described under the Upavisa Varga in**Rastarangani**[2],and **Rasratna Samuchchaya**[3] etc. and is being used successfully in the management of several diseases after proper Shodhana process. Kupilu was not mentioned in the "Brihat Trayee" texts of Ayurveda but later it was mentioned in different Nighantus with a number of synonyms. A drug described as Vishamustika is found in the Surasadi gana of Sushruta Samhita. But it is not botanically identified as S.nux-vomica.[4]Dalhana also mentioned it as Raja Nimba.[5] But it's uses in Ayurveda were mentioned from the period of "Vrinda Madhava" (9th A.D.). While the drug Vishamusti was mentioned in the English translation of Vrinda's Siddha Yoga edited by P. V. Tiwari, in 'Vatavyadhichikitsa'. Later different authors mentioned it with a number of synonyms like Visatinduka, Kupilu, Visamusti[3], Kakatinduka[6] Karaskara[7], Kulaka, Jalada, Garadruma[7,8] etc, which indicates the toxic nature of this tree. On the other hand, European countries started using Kupilufrom sixteenth century onwards; however it was not widely used in drugs but was mainly utilized to poison dogs, cats, crows, etc.[9]

Synonyms of Kupilu

Kuchelak,Kuchel,Kuchila,Kuchil,Vishtindu,Tindu, Tinduk,Vishtinduk,

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Karskar,Ramyaphala,Kupak,
Vishmushtika,Vishmushti,Kalkut.

According to Ayurved Kupilu's Gun – Karmadi :[10]

Rasa- Katu, tiktaGuna–LaghuVipaka- KatuVeerya-Ushna

Kupilu's effect on Tridosha:

Kapha Vata hara - Balances vata and kapha. It increases Pitta Dosha[11]

Classical categorization

Bhavprakash Nighantu[10]-Amradi varga, Karskar kul

Classification of plant

Botanical name –Strychnos nux vomica

- Kingdom-Plantae
- Order-Gentianales
- Family-Loganinaceae

Varieties -Strychnos wallichiana, Strychnos ignati

Phyto-chemical composition

The dried Nux vomica seeds contain 2.6%- 3% total alkaloids, out of which 1.25%-1.5% is strychnine, 1.7% is brucine, and the rest are vomicine and igasurine.[11] While other alkaloids are αcolubrine, β-colubrine, 3-methoxyicajine, protostrychnine, novacine, n-oxystrychnine, pseudostrychnine, isostrychnine, chlorogenic acid, and glycoside.[12]

Signs & symptoms of toxicity:[13]

Intact seeds are non poisonous as pericarp cannot be digested. With broken seeds the features are -Bitter taste.

Twitching and stiffness of muscles of face and neck.Strychnine convulsions are initially clonic (intermittent) and then tonic (sustained). Any stimulus like movements of patient, noise, touch, light or water immediately produces convulsions. Muscles are rendered stiff and rigid so that body is thrown into the form of an arch.[13]

1) Only head and heels touching ground (convexity upwards) - Ophisthotonous posture.
2) Only back touching (convexity downwards)- Emprosthotonous posture.
3) Arching sideways – Pleurosthotonous posture.

Chest becomes fixed, breathing difficult, cyanosis. Blood stained froth at nose and mouth. Rhisus sardonicus (Fixed monkey likegrin) and spasm of jaw musclesproduces locked jaw (trismus). Eyes are prominent and staring, withdilated pupils. Mind remains clear till end. It leads to painful death.

Causes of death:[13]

Medullary paralysis, asphyxia due to spasm of respiratorymuscles,exhaustion.

Fatal dose: [13]

2 crushed seeds (15-30 mg of strychnine)

Lethal dose: The lethal dose of strychnine is 30-120mg for humans, and for mice it is 0.98-2 mg/kg. [14]

Fatal period: 2 hours.[13]

Treatment:[13]

Shift patient to dark quiet room, anaesthetise the patient.

Antidote - barbiturates phenobarbitone sodium IV, other drugs as mephanesin, muscle relaxants,artificial respiration,symptomatic treatment,gastric lavage with tannic acid, strong tea or KMnO4

Jambir (citrus limon)

Description of Jambir in Samhita:

There is description of Jambir or Nimbuk varietiey in samhitas. It is described under Phala varga and Amla varga. In Charak Samhita, there is description of Matulung or Bijaura in Annapanvidhi Adhyay which is C. limon variety. Again in Sushrut Samhita there is description of Bijapura. While In Bhavprakash Nighantu, the varieties are as Mishta Nimbuk, Jambeera and Nimbuk.Where it is described as Rochana, Deepana, Pachana etc. After some years, in Ras shastra era it was used in Ras kalpa mainly as Bhavana dravya.

Classification of plant

Botanical name - Citrus limon

- Kingdom-Plantae
- Division-Magnolophyta
- Class-Magnoliopsida
- Order - Spanidales
- Family - Rutaceae
- Genus - Citrus
- Species - Limon

Synonyms in Sanskrit -Jambh, Jambir, Jaamphal, Nimbu, Nimbuka, Naaranga, Limpaka, Dantashatha, Airavata, Neebu (bigger var.).

F)Classification according to Ayurved

Charaka - Phala Varga, Amla varga[15]

Sushruta - Phala Varga[16]

Bhavprakash- Phala Varga[17]

Rajnighantu- Amla varga[18]

According to Ayurved Jambir's Gun – Karmadi :

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Rasa-Amla Guna-Teekshna, Laghu Veerya-UshnaVipaka-Amla

Effects on Doshas - It balances Vata and Kapha doshas.

Phytochemicals of C. limon

The chemical composition of C. limon fruithas not only been determined for the whole fruit but also separately for the pericarp, juice, pomace, and essential oil. Also the compositions of the leaves and the fatty oil extracted from C. limon seeds are also known.

Due to the large number of C.limon varieties, cultivars and hybrids, various research centres undertake the task of analyzing the chemical composition of the raw materials obtained from them.

The most important group of bioactive compounds in both C. limon fruit and its juice, determining their biological activity.

Flavonoids- Such as:

Flavonones - Eriodictyol, hesperidin, hesperetin, naringin

Flavones- Apigenin, diosmin.

Flavonols-Quercetin; and their derivatives

In the whole fruit, other flavonoids are additionally detected:

Flavonols - Limocitrin and spinacetin

Flavones- Orientin and vitexin

Some flavonoids, such as neohesperidin, naringin and hesperidin are characteristic for C.limon fruit. In comparison to another Citrus species, C. limon has the highest content of eriocitrin.[1]

Apathya

Introduction of pathya-pathya

The words Pathya–Apathya are very commonly used in Ayurved litreratures. This concept of Pathya and Apathya is stressed by all Acharyas in their Samhitas. One can find this term while describing about dravya, aahar,vyadhi, samprapti, ritucharya, dincharya etc. Ayurved is incomplete without this concept.These words are also well defined by Acharyas to know more about it. Pathya means do's and Apathya means don'ts .

When one says prevention is better than cure,the prevention is well achieved by Pathya-Apathya.

Ayurveda aims to keep a one's physical, mental, and adhyatmik aspects in balance. The person develops health problems and diseases when this equilibrium gets disturbed. For maintenance of this equilibrium Ahar,nidra,brahmacharya is the best way to follow which is mentioned as Trayoupstambh. Ahar is the most important amog all.Acharya Kashyapa has said that food is Mahabheshaja. In this series, pathya- kalpana is the basic and most powerful component while treating the disease. It is possible if one uses diet according the Tridosha, Deha prakriti, Satmya,Desh,Kal,Satva etc.

Nirukti

Pathya is derived from the root word "Patha", which means "a way or channel."

Definition

According to Charakacharya, Pathya, which is the Aahar-vihar that calms the mind, is beneficial to the body, and provides nutrients. Apathya, is exactly opposite of Pathya.[20]

The aharadi dravya which do not harm the path i.e. strotas,and which calms the mind is called as pathya.While apathya is that aharadi dravya which harms the path anddisturbs peace of mind and body.

Pathya -Apathya include:

- Foods indicated for healthy people.
- Foods contraindicated for healthy people.
- Pathya-Apathya towards the patient.

Materialand methodology

1. Collection of study material.
2. Identification and authentication of study material.
3. Analytical study of Kupilu beej churna and Neembu swaras.
4. Preparation of Kupilu beej churna
5. Animal study

Animal study

It was an experimental type of study. Study was done in the college of pharmacy.Duration of the study was 7 days for acute toxicity.Animals were housed under standard laboratory conditions i.e. temperature of 24±1°Cand 30-70% humidity and normal dark and light cycle of 12 hrs. Food given was chow pellets and water. Animals were acclimatised to laboratory conditions before the experiment. The experiment was carried out between 10:00 and 17:00h.

The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).Before starting animal experiment, the permission of incharge of institute was taken where

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the animal experiment was done. Approval of Institutional Biosafety committee (IBSC) and Institutional Animal Ethics Committee (IAEC) was taken.

Method of experiment in Albino mice was procured by considering

A. Inclusion criteria

Healthy Albino mice of either sex were considered.
Mice weighing about 25 to 30 g were included.
Age between 6 to 8 weeks were selected.

B. Exclusion criteria

Mice less than 25 g and more than 30 g.
Pregnant and diseased mice.
Mice which were under trial of other experiments.

The study was done under two stages:

Stage 1–LD 50 doseof Kupilu /strychnine in swiss albino mice was searched in literature such as books,research articles. 1/10 th of the LD50 dose was given orally to produce acute oral toxicity according to OECD guidelines 425.

Stage 2-Apathya i.e. Jambir Swaras was given orally after acute toxicity produced by Kupilu beej churna.

Procedure

Collection of drugs:

- Kupilu beej – Kupilu beej were collected from Ayurvedic raw material store.
- Jambeer/Neembu for it's swaras – Collected from vegetable market.

Grouping:

- For the experimental study, 18 Albino mice of both sex, weighing between 25-30g, and age between 6 to 8 weeks were selected.
- Each Albino mice was weighed accurately, and marked by marker pen.
- The mice were distributed in 3 equal groups, comprising 3 males and 3 females in each group.

Preparation of mice:

- The micewere on normal animal diet.
- They were allowed to acclimatise for laboratory temperature i.e. for room temperature.

Dosing:

Calculation of the dose-

Kupilu- LD 50 dose of Kupilu /strychnine in swiss albino mice was searched in literature such as books,research articles, which is 0.98 -2 mg/kg.[36]

1/10 th of the LD50 dose was given orally to produce acute toxicity.

Jambir swaras- No such exact dose information for apathya is available, the swaras was given as in 1 to 1/2 drop i.e. up to 0.05ml.

Drug administration:

Group I – The mice of this group were kept on normal diet and habitat.Neither envenomation nor any drug administration was done.

Group II- The drug Kupilu beej churna suspended in distilled water, was given to the mice orally by gavage, after calculating the LD 50. After dosing, animals were observed for toxicity symptoms and the observations were noted.

Group III-To this group, toxicity was developed as group II, and then Jambir swaras was given orally. Again symptoms were observed and noted.

Criteria for assessment

a)The parameters for observation of toxic symptoms were asfollows:

1)Respiratory rate2)Heart Rate
3)Tremors 4)Convulsions
5)CNS Depression 6) Muscle spasm
7) Muscle relaxation 8) Urination
9) Salivation 10)Sedation
11)Lethargy

b) Haematological tests:

Hemoglobin, RBC, WBC and platelets were considered for analysis. These procedures were performed by cell machine (Hematology counter analyser),of three animals of each group.SGOT was performed by Automated biochemical analyser. Blood glucose was done by Glucometer.

III. OBSERVATIONS AND RESULT

The animal experiment was carried out on Albino mice in pharmacy college. For experiment the healthy Swiss Albino mice of 25-30 g of weight, age 6-8 weeks, of either sex, non pregnant, and which were not under any experimental study were selected. Three groups of 6 animals in each, containing 3 males and 3 females were created. The three groups were as control group (Group I), disease control group(Group II), and test control group (Group III),

To the first group i.e. control group, no drug was given. Their normal behaviour was observed.

To the second group i.e. disease control group, the Kupilu beej churna mixed in distilled water was given orally. The dose was given as 1/10

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th of the dose of the LD 50 of Albino mice to produce acute oral toxicity. The toxicity symptoms were observed and noted. The observations were done for 6-8 hrs by 1/2 an hourly. Animals were observed individually after dosing continuously during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4-6 hours. However, the duration of observation was not being fixed rigidly. All observations were systematically recorded with individual records being maintained for each animal in the daily observation record format for 7 days.

The parameters to observe were respiratory rate, heart rate, tremors, convulsions, CNS depression,muscle spasm, muscle relaxation, lethargy, urination, salivation and sedation.

To the third group i.e. test control group, as Apathya,Jambir swaras was given orally after Kupilu beej toxicity. Again the observations were made and noted.

Along with these observations haematological tests were done in all the three groups of three animals of each group. CBC, SGOT and blood glucose were done.

1.Respiratory rate:

Swiss albino mice of group I,II and III were i.e. control group, disease group and test control group were observed for respiration for 2 min.

Table no. 18-Statistical analysis of Respiratory rate

Sr. No.	Group I	Group II	Group III
1	115	175	230
2	110	180	240
3	95	177	200
4	140	170	214
5	100	186	190
6	140	160	220
Mean ± SD	**116.67 ± 19.41**	**174.67 ± 8.94**	**215.67 ± 18.57**
SEM	**7.92**	**3.65**	**7.58**
Range	**95 – 140**	**160 – 186**	**190 – 240**
95% CI	**96.30 – 137.04**	**165.29 – 184.05**	**196.18 -235.15**
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is < 0.0001, considered extremely significant.		
	If the value of q is greater than 3.674 then the P value is less than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-58.000	8.693	< 0.001, highly significant
Group I vs Group III	-99.000	14.839	< 0.001, highly significant
Group II vs Group III	-41.000	6.145	< 0.01, very significant

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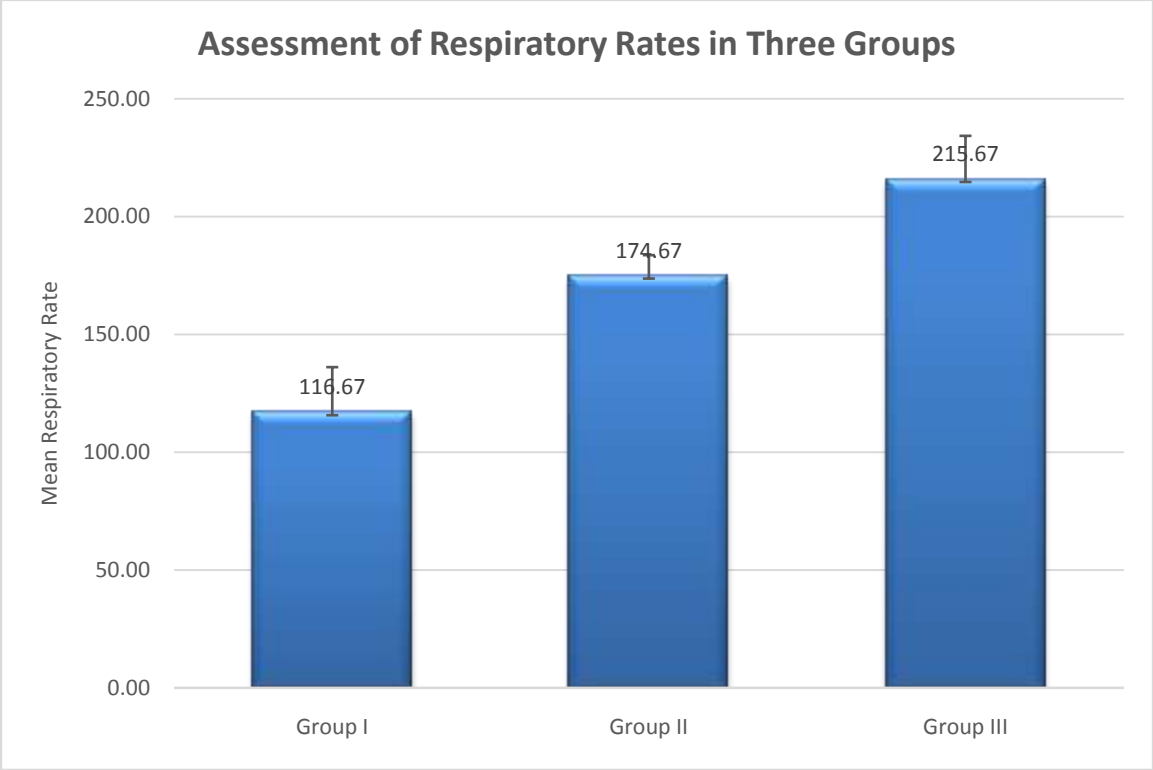

Chart no.1- Graphical presentation of Statistical analysis of Respiratory rate

2. Heart Rate

Swiss albino mice of group I, II and IIIwere i.e. control group, disease group and test control group were observed for heart rate for 2 min.

Table no-19 Statistical analysis of Heart rate

Sr. No.	Group II	Group II	Group III
1	270	460	568
2	330	430	456
3	340	456	567
4	370	523	567
5	230	421	547
6	350	321	456
Mean ± SD	**315.00 ± 53.57**	**435.17 ±66.37**	**526.83 ± 55.43**
SEM	**21.87**	**27.10**	**22.63**
Range	**230 – 370**	**321 – 523**	**456 – 568**
95% CI	**258.77 – 371.23**	**365.50-504.83**	**468.65 –585.01**
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is < 0.0001, considered extremely significant.		

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	If the value of q is greater than 3.674 then the P value is less than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-58.000	5.012	< 0.001, highly significant
Group I vs Group III	-99.000	8.835	< 0.001, highly significant
Group II vs Group III	-41.000	3.823	< 0.01, very significant

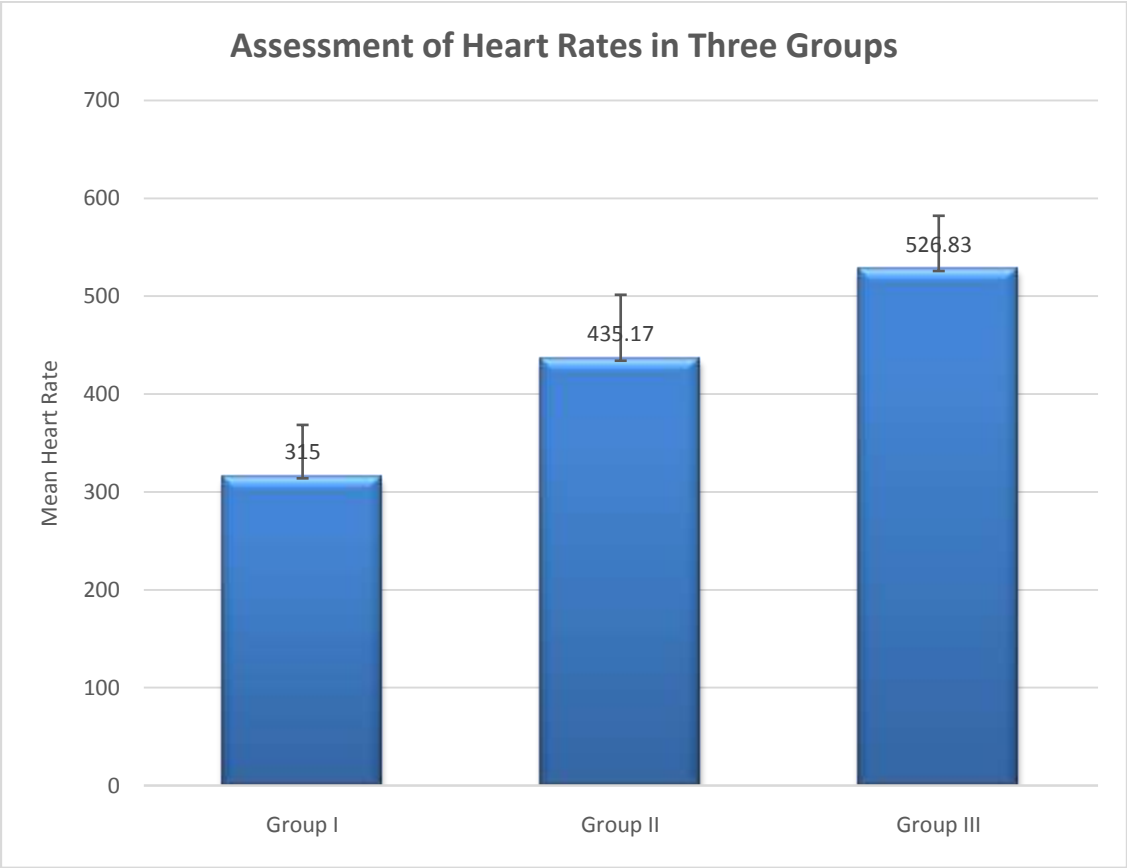

Chart no.2- Graphical presentation Statistical analysis of heart rate.

3.Tremors:

Swiss albino mice of group I,II and III i.e. disease group and test control group were observed for number of tremors for 5 min.

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Table no. 20- Statistical analysis of Tremors

Sr. No.	Group I	Group II	Group III
1	0	22	36
2	0	12	40
3	0	14	34
4	0	18	29
5	0	18	28
6	0	22	34
7	0	17	32
Mean ± SD	**00**	**17.57 ± 3.74**	**33.29 ± 4.11**
SEM	**00**	**1.412**	**1.55**
Range	**00**	**12 – 22**	**28 – 40**
95% CI	**00**	**14.12 – 21.03**	**29.48 – 37.09**
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is < 0.0001, considered extremely significant.		
	If the value of q is greater than 3.609 then the Pvalue is lessthan 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-17.571	14.496	< 0.001, highly significant
Group I vs Group III	-33.286	27.459	< 0.001, highly significant
Group II vs Group III	-15.714	12.964	< 0.001, highly significant

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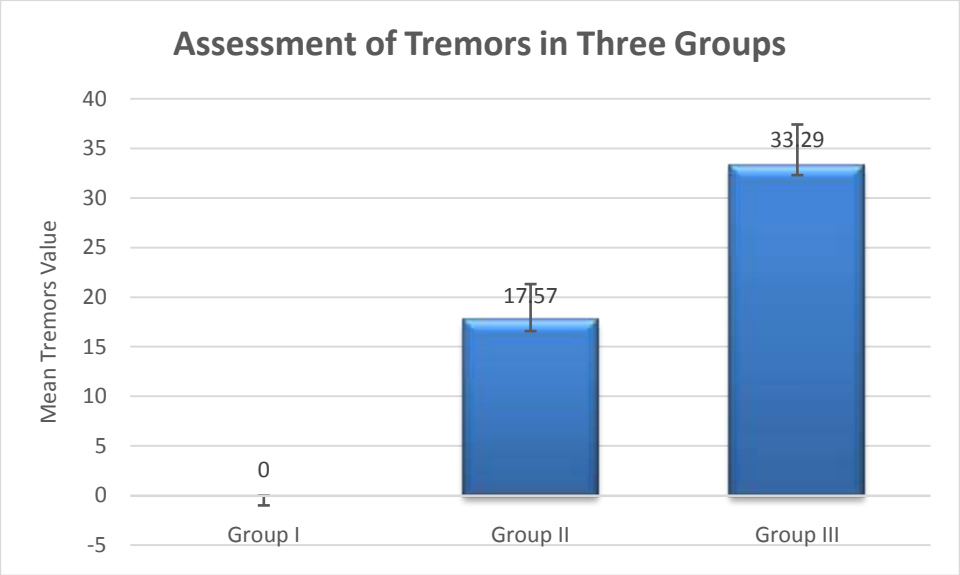

Chart no.3- Graphical presentation of Statistical analysis of tremors

4. Convulsions Swiss albino mice of group I,II and III were i.e. disease group and test control group were observed for number of convulsions and latency was measured.

Table no 21-Statistical analysis of Convulsions

Sr. No.	Group I	Group II	Group III
1	0	10	22
2	0	12	23
3	0	18	31
4	0	20	29
5	0	15	32
6	0	19	29
Mean ± SD	**00**	**15.67 ± 4.03**	**27.67 ± 4.18**
SEM	**00**	**1.647**	**1.706**
Range	**00**	**10 – 20**	**22 – 32**
95% CI	**00**	**11.43 – 19.90**	**23.28 – 32.05**
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is < 0.0001, considered extremely significant.		

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	If the value of q is greater than 3.674 then the P value is less than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-15.667	11.444	< 0.001, highly significant
Group I vs Group III	-27.667	20.210	< 0.001, highly significant
Group II vs Group III	-12.000	8.766	< 0.001, highly significant

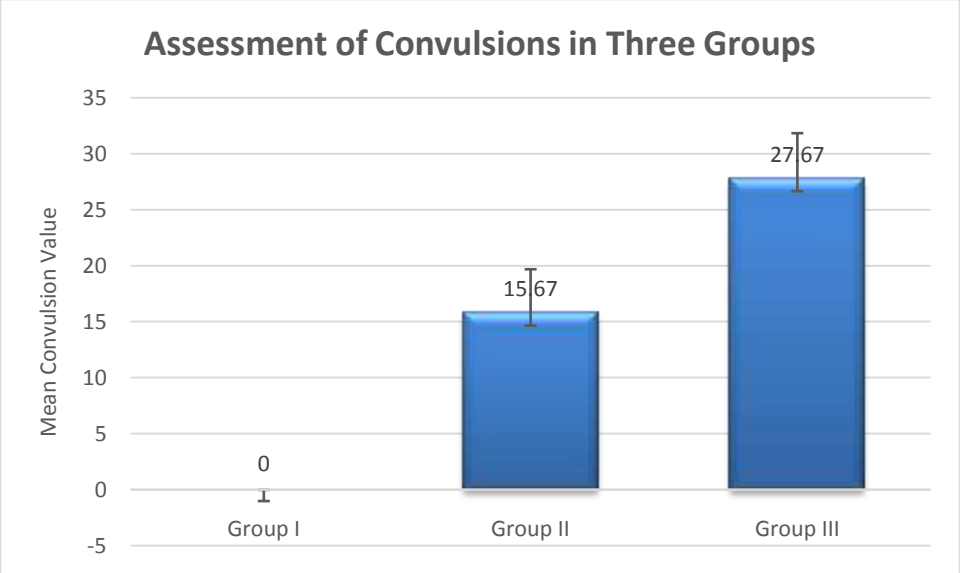

Chart no.4- Graphical presentation of Statistical analysis of convulsions

5. CNS Depression

CNS Depression in mice was evaluated by using Actophotometer apparatus. It was 30×30×30 cm in dimension. The two photocells were fixed 3 cm above the floor. Each wall of the cage had 3 equidistant holes in a horizontal array, 7 cm above the floor. Through the holes 3 light beams were passed from all sides of the apparatus. They crossed in the centre of the cage. There were the infra-red filters to minimize the effect of light on behaviour. The number of interruptions of the light beams were recorded on digital counters in the instrument automatically.

Procedure

Each mouse was kept for 5 minutes in the action cage. If mouse started the exploratory action, then the beam of light was interrupted. Each interruption was picked up automatically by the digital recorder in the instrument and was counted. If the animal stopped the locomotor action, the digital counter stopped recording. Again, when animal started walking, the action was recorded. The "Walks" were defined as the number of durations the mouse moved with all four feet in the space between two opposite walls of the cage. The total duration consumed in walking was recorded as above

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Actophotometer Readings:

Table no 22: Statistical analysis of CNS depression

	Group I	Group II	Group III
Before	257.50 ± 20.60	261 ± 22.34	257 ± 20.68
After	259.17 ± 20.24	185 ± 12.72	123.67 ± 10.11
After	The p value is < 0.0001, considered extremely significant.		
	One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	74.170	1105.7	< 0.001, highly significant
Group I vs Group III	135.50	2019.9	< 0.001, highly significant
Group II vs Group II	61.330	914.25	< 0.001, highly significant

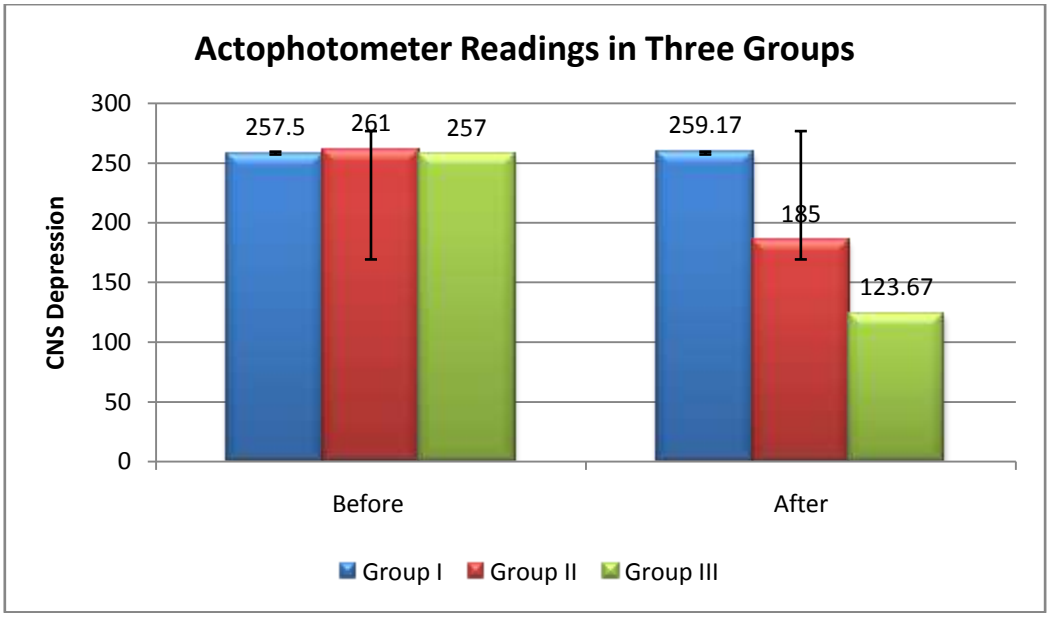

Chart no.5- Graphical presentation of Statistical analysis of CNS depression

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6.Muscle Relaxation:

Muscle Relaxant Activity using Rotarod Apparatus

Mice were placed on the slowly rotating rod [5 rotations per minute (rpm)] against the direction of the rotation. The rod then progressively accelerated up to a speed of 25 rpm over a duration of 5 minutes. Four trials a day were performed for three consecutive days with a trial ending when either an animal fell off or the maximum trial time of five minutes had elapsed. Inter-trial intervals of 3 to 5 minutes were employed between trials. Latency to fall was recorded.

Table no 23. Statistical analysis ofMuscle relaxation

Sr. No.	Group I	Group II	Group III
1	297	189	185
2	242	200	192
3	276	280	270
4	255	260	249
5	242	190	184
6	245	198	192
Mean ± SD	259.5 ± 22.46	219.5 ± 39.86	212 ± 37.54
SEM	9.17	16.27	15.33
Range	242 – 297	189 – 280	184 – 270
95% CI	235.93 – 283.07	177.66 – 261.34	172.60 – 251.40
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is 0.0626, considered not quite significant.		
	If the value of q is greater than 3.674 then the P value is lessthan 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	40.000	2.868	> 0.05, not significant
Group I vs Group III	47.500	3.405	> 0.05, not significant
Group II vs Group III	7.500	0.5377	> 0.05, not significant

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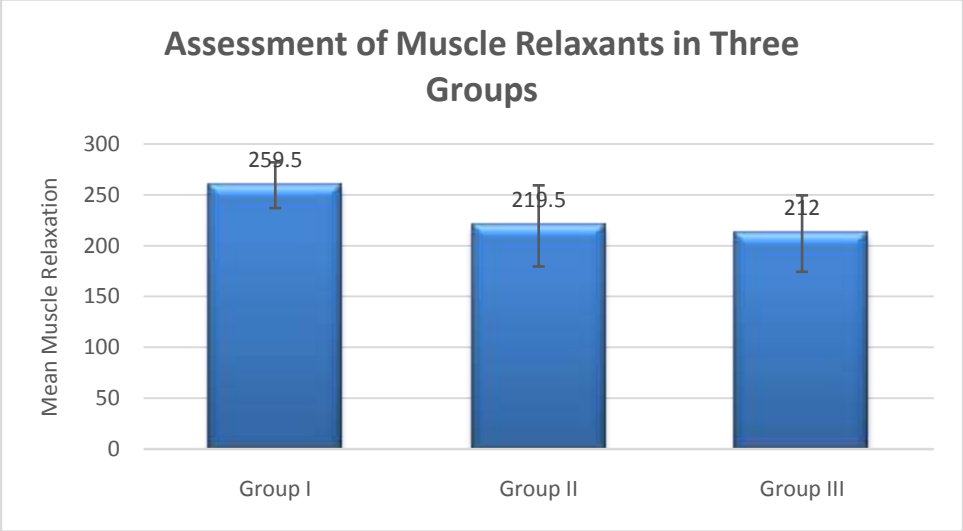
Chart no.6- Graphical presentation of Statistical analysis of muscle relaxation



7.Muscle spasm: Swiss albino mice of group I,II and III were i.e. disease group and test control group were observed for writhe (Twitching in abdomen) for 5 minutes.

Table no. 24- Statistical analysis of Muscle spasm

Sr. No.	Group I	Group II	Group III
1	0	31	33
2	0	26	24
3	0	40	40
4	0	46	45
5	0	36	38
6	0	32	36
Mean ± SD	00	35.17 ± 7.11	36 ± 7.13
SEM	00	2.903	2.91
Range	00	26 – 46	24 – 45
95% CI	00	27.70 - 42.63	28.52 – 43.48
Inter-Group Comparison	One-way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparisons Test		
	The P value is < 0.0001, considered extremely significant.		

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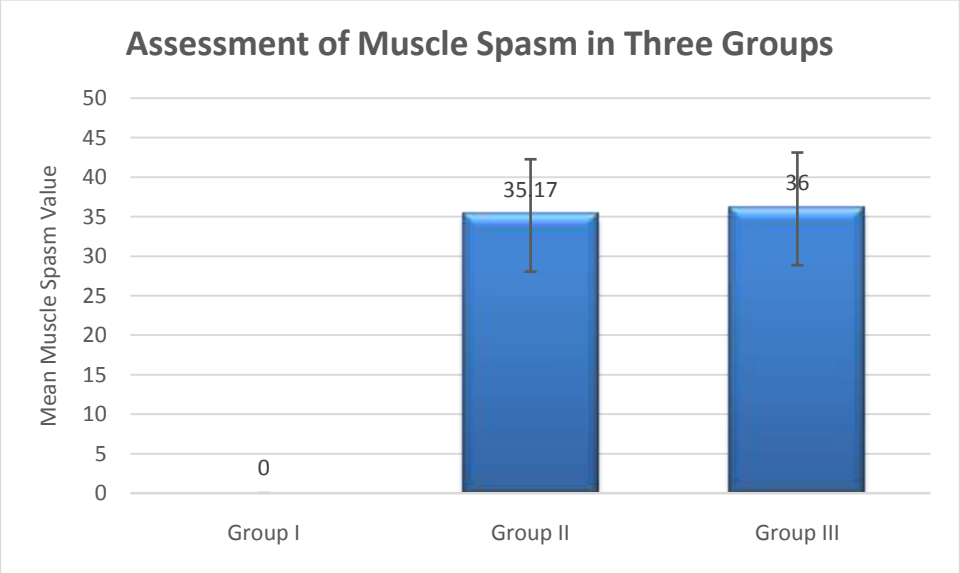
	If the value of q is greater than 3.674 then the P value is less than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-35.167	14.819	< 0.001, highly significant
Group I vs Group III	-36.000	15.170	< 0.001, highly significant
Group II vs Group III	-0.8333	0.3512	> 0.05, not significant

Chart no.7- Graphical presentation of Statistical analysis of muscle spasm



8. Haematological Parameters

Blood withdrawal using Retroorbital route

Animals were anaesthetized with mild ether anaesthesia. A drop of proparacaine topical ophthalmic anaesthetic in each eye was used to minimize discomfort .The animal was held by the back of the neck and the loose skin of the head is tightened with thumb and middle finger to keep the animal stable. The tip of the capillary tube was placed at the medial canthus of the eye under the nictitating membrane. With a gentle thrust and rotation motion past the eyeball the tube was enter the slightly resistant sinus membrane. The eyeball itself remains uninjured. As soon as the sinus was punctured, blood enters the tubing by capillary action. Around 1mL of blood was collected, the tube was withdrawn and slight pressure with a clean gauze pad on the eye was used to ensure haemostasis. The blood was then used for estimation of haematological parameters.

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Blood Haemoglobin:

Table no 25: Statistical analysis of Blood Haemoglobin

	Group I	**Group II**	**Group III**
Mice 1	15.4	15.4	13.4
Mice 2	14.4	13.4	14.4
Mice 3	12.8	12.8	12.8
Mean ± SD	14.2 ± 1.31	13.87 ± 1.36	13.53 ± 0.81
SEM	0.76	0.79	0.47
Range	12.8 – 15.4	12.8 – 15.4	12.8 – 14.4
95% CI	10.94 – 17.46	10.49 – 17.25	11.53 – 15.54
Inter-Group Comparison	**One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test**		
	The p value is 0.7926, considered not significant.		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	0.3333	0.4864	> 0.05, not significant
Group I vs Group III	0.6667	0.9728	> 0.05, not significant
Group II vs Group II	0.3333	0.4864	> 0.05, not significant

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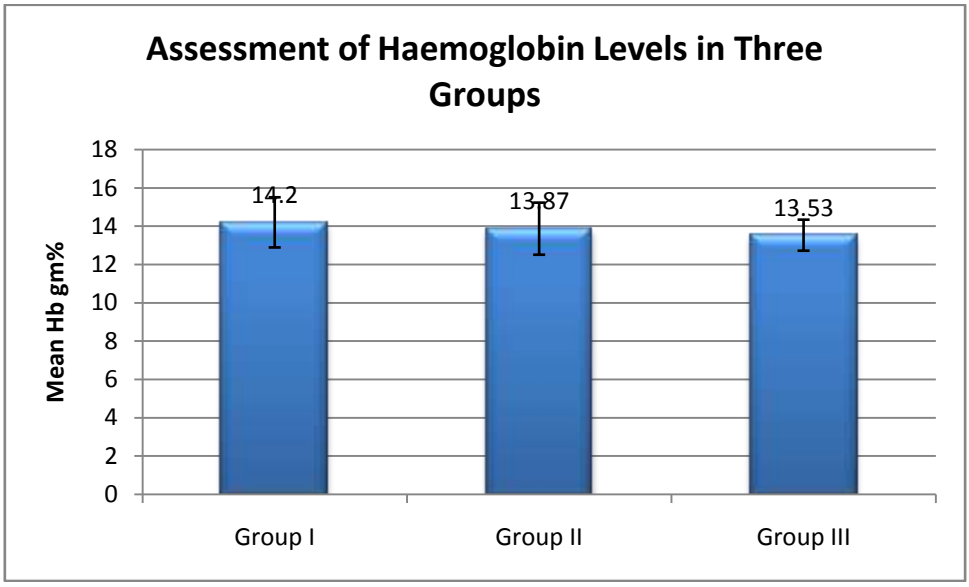

Chart no.8- Graphical presentation of Statistical analysis ofhaemoglobin

RBCs:

Table no 26 - Statistical analysis of RBCs

	Group I	**Group II**	**Group III**
Mice 1	8.67	9.60	7.40
Mice 2	9.54	6.30	7.30
Mice 3	7.90	6.90	8.50
Mean ± SD	8.70 ± 0.82	7.6 ± 1.76	7.73 ± 0.67
SEM	0.47	1.015	0.38
Range	7.9 – 9.54	6.9 – 9.6	7.4 – 8.5
95% CI	6.67 – 10.74	3.23 – 11.97	6.08 – 9.39
Inter-Group Comparison	**One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test**		
	The p value is 0.5015, considered not significant.		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	1.103	1.614	> 0.05, not significant
Group I vs Group III	0.9700	1.419	> 0.05, not significant
Group II vs Group II	-0.1333	0.1950	> 0.05, not significant

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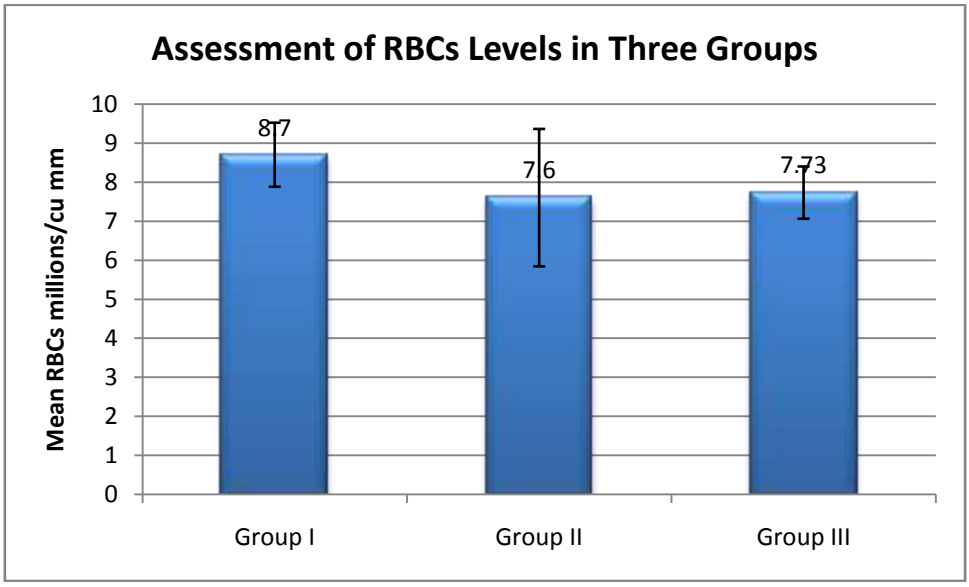

Chart no.9- Graphical presentation of Statistical analysis of RBCs

Platelets:

Table no 27- Statistical analysis of Platelets

	Group I	**Group II**	**Group III**
Mice 1	203	289	279
Mice 2	230	243	243
Mice 3	256	287	283
Mean ± SD	229.67 ± 26.50	273 ± 26	268.33 ± 22.03
SEM	15.30	15.01	12.72
Range	203 – 256	243 – 289	243 – 283
95% CI	163.83 – 295.51	208.41-337.59	213.60 – 323.06
Inter-Group Comparison	**One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test**		
	The p value is 0.1433, considered not significant.		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-43.333	3.011	> 0.05, not significant
Group I vs Group III	-38.667	2.687	> 0.05, not significant
Group II vs Group II	4.667	0.3243	> 0.05, not significant

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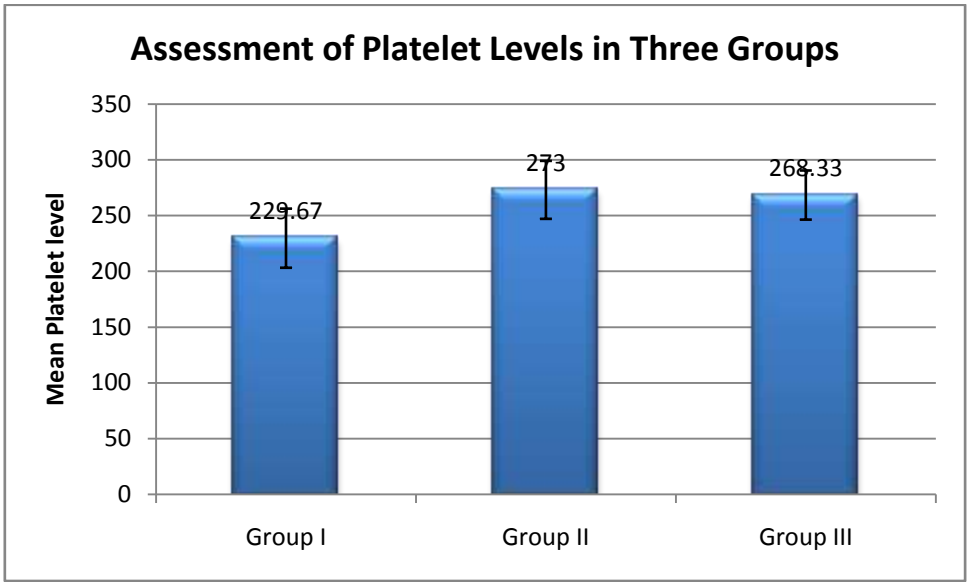

Chart no.10- Graphical presentation of Statistical analysis of platelets

SGOT:

Table no. 28- Statistical analysis of SGOT

	Group I	**Group II**	**Group III**
Mice 1	115	128	130
Mice 2	130	119	145
Mice 3	130	119	124
Mean ± SD	125 ± 8.66	122 ± 5.20	133 ± 10.82
SEM	5.00	3.00	6.25
Range	115 - 130	119 – 128	124 – 145
95% CI	103.49 – 146.52	109.09 – 134.91	106.13 – 159.87
Inter-Group Comparison	**One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test**		
	The p value is 0.3329, considered not significant.		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	3.000	0.6082	> 0.05, not significant
Group I vs Group III	-8.000	1.622	> 0.05, not significant
Group II vs Group II	-11.000	2.230	> 0.05, not significant

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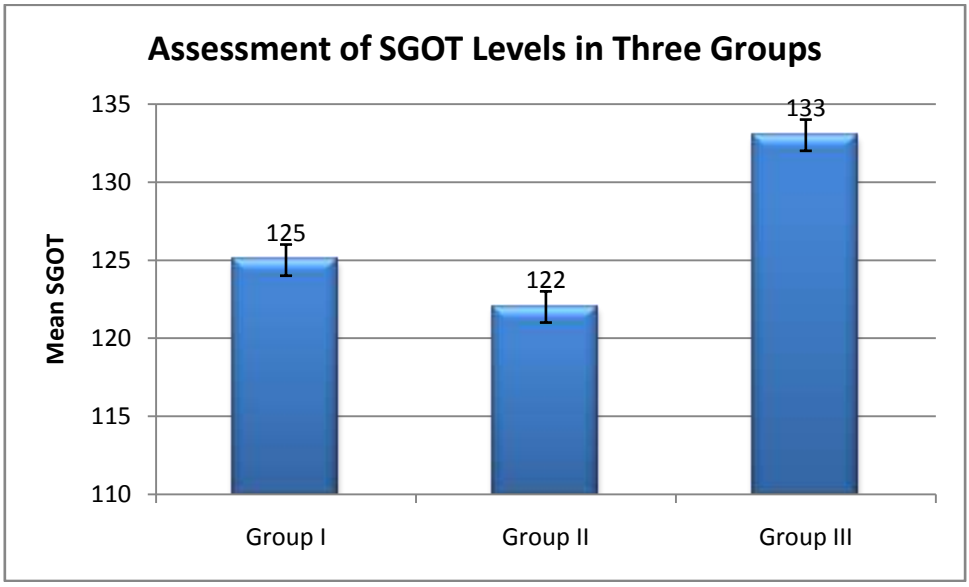

Chart no.11- Graphical presentation of Statistical analysis of SGOT

Glucose:

Table no29- Statistical analysis of Glucose

	Group I	**Group II**	**Group III**
Mice 1	135	161	165
Mice 2	145	153	154
Mice 3	156	145	149
Mean ± SD	145.33 ± 10.50	153 ± 8.00	156 ± 8.19
SEM	6.06	4.62	4.73
Range	135 – 156	145 – 161	149 – 165
95% CI	119.24 – 171.43	133.13 -172.87	135.66 – 176.34
Inter-Group Comparison	**One Way Analysis of Variance (ANOVA) Tukey-Kramer Multiple Comparison Test**		
	The p value is 0.3837, considered not significant.		
	If the value of q is greater than 4.339 then the p value is lesser than 0.05.		
	Mean Difference	**q**	**p value**
Group I vs Group II	-7.667	1.481	> 0.05, not significant
Group I vs Group III	-10.667	2.060	> 0.05, not significant
Group II vs Group II	-3.000	0.5793	> 0.05, not significant

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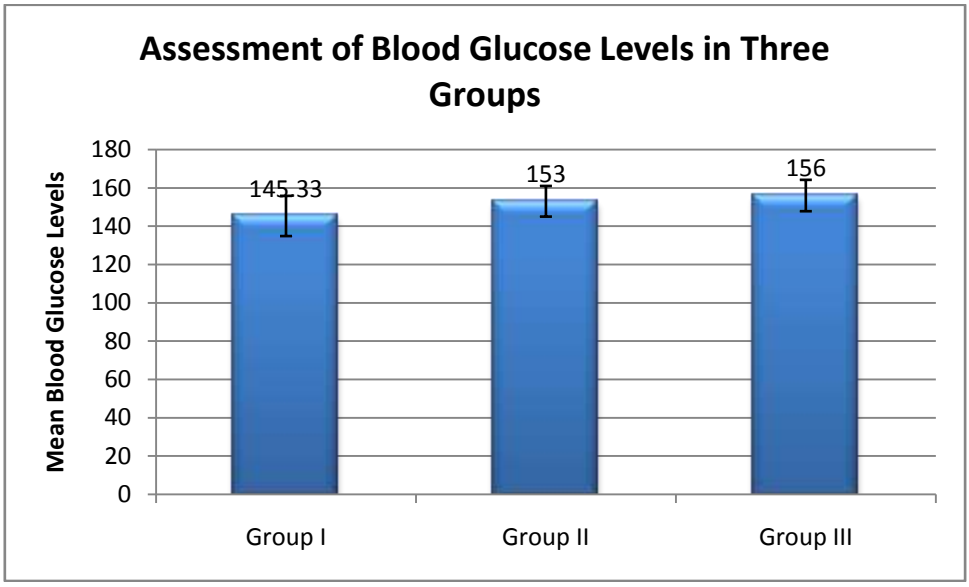

Chart no.12- Graphical presentation of Statistical analysis of glucose

Other parameters observed while performing experimental study:

A) Disease control group (Kupilu beej churna) / group II

GROUP	SEX	M	M	M	F	F	F
DISEASE CONTROL GROUP (KUPILU)	SEX MARKING	DM1	DM2	DM3	DF1	DF2	DF3
URINATION		_	_	_	+	_	_
SALIVATION		_	+	_	_	_	_
SEDATION		_	_	_	_	_	_
LETHARGY		++	+	+	++	++	++

Table no 30- Table of Gradation of parameters ofgroup II

Gradings:
Low : + **Moderate: ++** **High : +++**

Inference- From the above table it was observed that after Kupilu beej churna oral administration, there were no changes in urination, salivation, sedation while there was moderate increase in lethargy.

A) Test control group

Test control (Kupilu beej churna + Jambir swaras)/ group III

GROUP	SEX	M	M	M	F	F	F
Test control (Kupilu beej churna + Jambir swaras)	MARKING	TM1	TM2	TM3	TF1	TF2	TF3
URINATION		_	+	_	_	_	_
SALIVATION		_	_	_	_	_	_
SEDATION		_	_	_	+	_	_
LETHARGY		++	++	+++	+++	++	+++

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Table no 31- Table of Gradation of parameters of group III
Gradings:
Low : + **Moderate : ++** **High : +++**

Inference- From the above table it was observed that after administration of Kupilu beej churna followed by Jambir swaras in group III, again there were no changes in urination, salivation, sedation,while there was more increase in lethargy than group II.

Observations :

The animal experiment was done in 3 groups of Albino mice comprising 6 animals in each group of either sex , with 3 males and 3 females.

The observations were made and noted during the experiment according to the parameters.

The parameters for observation were respiratory rate, heart rate,tremors,convulsions,CNS depression, muscle relaxation,muscle spasm urination, salivation,sedation, lethargy. The hamatological parameters that were observed were haemoglobin, RBC,platelets, SGOT and glucose. During the experiment it was observed that after Kupilu beej churna oral administration to the group I, there was marked increase in respiratory rate, heart rate, tremors, convulsions, CNS depression, muscle relaxation,muscle spasm and some moderate increase in lethargy. While there were no changes in urination, salivation and sedation. The most important finding was the convulsions, which began after the tremors. During convulsions, muscles were rigid but typical arch formation of body was not found such as ophisthotonus, emprosthotonous and pleurosthotonus.

To the third group i.e. test control group, as Apathya,Jambir swaras was given orally after Kupilu beej toxicity. Again the observations were noted.It was observed that the parameters were increased, specially the convulsions i.e. the frequency, time duration, latency and severity of convulsions. Also there was marked increase in respiratory rate, heart rate, tremors, CNS depression, muscle relaxation,muscle spasm and some more increase in lethargy than group II. While there were no changes in urination, salivation and sedation.

Along with these observations haematological tests were done in both the group. The parametres considered were haemoglobin, RBC, platelets, SGOT and blood glucose. There were no significant differences in all the 3 groups.

The statistics was applied in all the three groups and the data was compared, using software to draw the conclusion of the experiment. The mean, SEM,SD, class interval were drawn for inter-group comparison. And the p value was considered for assessment. The p values of the each parameter is as follows-

1.Respiratory rate: $p < 0.0001$
2.Heart rate $p < 0.0001$
3.Tremors $p < 0.0001$
4.Convulsions$p < 0.0001$
5.CNS depression$p < 0.0001$
6.Muscle spasm$p < 0.0001$
7.Muscle relaxation $p = 0.0626$
8.Urination -Not observed in group I
One mice of group II
One mice of group II
9.Salivation - Not observed in group I
One mice of group II
Not observed in group III
10.Sedation Not observed in group I
Not observed in group II
One mice of group II

Haematological parameters
Haemoglobin $p = 0.7926$
RBC $p = 0.5015$
Platelets $p = 0.1433$
SGOT $p = 0.3329$
Blood glucose $p = 0.3837$

IV. DISCUSSION

The toxicity by drug , plant or by any animal is always the subject of concern. The toxicity may be accidentaly or with sheer purpose to eliminate human or animal. The concern increases when it leads to the sudden death. Hence the immediate treatment is anticipated. The survival of the victim is always depends upon the specific toxin, it's dose,time since toxicity, route of administration, age etc. After the immediate treatment, if the person survives,it's after effects or adverse effects are seen on the patient.Where the morbidity is common. The after treatment includes recovery of the patient from morbidity.

Where Ayurveda believes in Swasthasya swastha rakshanam.Where Vaidya has to treat the patient to achieve, Samdosha,samagni,samdhatu mal kriya.

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For Vish Chikitsa 24 types of treatment i.e.Chaturvinshati upakramaare recommended by Charakacharya.The treating doctor chooses the best treatment for the patient. Along with the any type of treatment,there is always recommendation of do's and don'ts in Aahar -Vihar for the best results. That is called as Pathya-Apathyawhich is elaborately discussed in Samhitas. As Apathya increases the symptoms and complications of the disease, henceit should be avoided. Likewise Apathya is also recommended in VishChikitsa.

Such Apathya is recommended in the bookRasajalnidhi whichhas been choosen for research study. Rasajalnidhiis the book which is also called as ocean of Indian chemistry, medicine and alchemy. This book is compiled in Sanskrit by Kaviraj Bhudeb Mookerji, published by Shrigokul Mudranalaya , Varanasi in 1984.The mentioned verse isunder the heading of Vishasevane apathyam. Where Apathya is recommended for intoxicated person.[1]

This shloka means if someone is toxicated, or though the person is accustomed to the poison, he should not consume Katu, Amla,Lavan Rasa and Tailam. Also, he should not have breeze, sunlight and he should not sleep in the day time.

It tells that if we do not follow this, the toxic effects of any drug will increase. So, rationale is that, we can advise to follow these rules to the intoxicated person to improve from it.

The literature study was done from various texts, articles, research papers. After drug authentication, the animal experiment was carried out on Albino mice in pharmacy college. For experiment the healthy Swiss Albino mice of 25-30 g of weight, age 6-8 weeks, of either sex, non pregnant, and which were not under any experimental study were selected. Three groups of 6 animals in each, containing 3 males and 3 females were created. The three groups were as control group, disease control group and test control group.

To the first group i.e. control group, no drug was given. Their normal behaviour was observed.

To the second group i.e. disease control group, the Kupilu beej churna mixed in distilled water was given orally. The dose was given orally as 1/10 th of the dose of the LD 50 of strychnine of Albino mice as per OECD guidelines 425. The toxicity symptoms were observed and noted. The observations were done for 6-8 hrs by 1/2 an hourly. Animals were observed individually after dosing continuously during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4-6 hours. However, the duration of observation was not being fixed rigidly. All observations were systematically recorded with individual records being maintained for each animal in the daily observation record format for 7 days.

The parameters to observe were respiratory rate, heart rate, tremors,convulsions, CNSdepression,muscle spasm, muscle relaxation, lethargy,urination, salivation andsedation.From the observations it is concluded that after Kupilu beej churna oral administration, there was marked increase in respiratory rate, heart rate, tremors, convulsions, CNS depression, muscle spasm, muscle relaxation and some moderate increase in lethargy. While there were no changes in urination, salivation and sedation. Urination was found in only one mouse and in another mouse salivation was observed.The most important finding was the convulsions, which began after the tremors. During convulsions, typical arch formation of body was not found such as ophisthotonus, emprosthotonous and pleurosthotonus.

To the third group i.e. test control group, as Apathya,Jambir swaras was given orallyafter Kupilu beej toxicity. Again the observations were noted.It was observed that the parameters were increased,specially the convulsions i.e. the frequency,time duration,latency and severity of convulsions. Alsothere was marked increase in respiratory rate, heart rate, tremors, CNS depression, muscle relaxation,muscle spasm and some more increase in lethargy than group II. While there were no changes in urination, salivation, sedation. Urination was found in only one mouse and in another mouse sedation was observed.

Along with these observations haematological tests were done in both the groups. Haemoglobin, RBC, platelet, SGOT and blood glucose were considered for analysis. There were no significant differences in all the three groups.

V. CONCLUSION

Present dissertation entitled, "In vivo study of Apathya in Kupilu beejpoisoning in Albino mice" was carried to study the effect of Aathya in toxicity.

Kupilu beej churna was used as a toxin to produce toxicity and Jambir swaras was used as Amla ras and as an Apathya.

LD 50 of Kupilu /strychnine: LD 50 of Kupilu /strychnine in swiss albino mice was

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searched in literature such as books,research articles, which is 0.98 -2 mg/kg.[14]

1/10 th of the LD50 was given orally to produce acute toxicity.

Effect of Kupilu as a toxin: The toxic effects of Kupilu was seen as increase Respiratory rate, heart rate and occurrence of tremors, convulsions and CNS depression, muscle spasm,muscle relaxation and lethargy, indicating the potential toxic effects of the drug. While there were no changes in urination, salivation and sedation.

Effect of Jambeer swaras: After giving Jambeer swaras as an Apathya there were significant increase in parameters of poisoning which were already produced by Kupilu. It was observed that there was increase in respiratory rate, heart rate, tremors, convulsions,CNS depression, muscle spasm and muscle relaxation. While there were no changes in urination, salivation and sedation.

Stastically all the three groups were compared and the assessment was done.

The p value ofrespiratory rate, heart rate, tremors, convulsions,CNSdepression,muscle spasm and muscle relaxation was less than 0.0001, which were highly significant.

While p value of muscle relaxation was0.0626 which was not quite significant.

And the p values ofhaematological parameters were as, Haemoglobin 0.7926, RBC0.5015, Platelets 0.1433,SGOT 0.3329, Blood glucose 0.3837 which were considered as not significant.

While there were no remarkable changes in urination, salivation and sedation in all the three groups.

So after considering all the data, the null hypothesis is rejected and the alternate hypothesis is accepted.

So it is concluded that the study suggests the Apathya or Jambeer swaras increases the toxicity.

The rejection of null hypothesis in multiple assessment criteria with p values less than 0.0001 indicates a statistically significant impact of Jambeer swaras.

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