Original Article

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Assessment of Acute and 28-Days Repeated Dose Sub-Acute Toxicity Study of Selected Ultra-Diluted Preparations in Wistar Rats

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Abstract

Objective: The aim of this work was to investigate the acute and sub-acute toxicity studies of the homeopathic drugs, causticum, calcerea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja in experimental models. *Materials and Methods:* Animals were divided into groups (n=5). Homeopathic drugs were administered to rats for 14 and 28 days to assess the toxicological profile. Acute and sub-acute studies were carried out according to OECD 425, 407 guidelines. Behavioural parameters and mortality rate were assessed in acute toxicity study. The sub-acute was performed for a study period of 28 days and afterwards animals were sacrificed to carry out biochemical and haematological estimations. The histopathological analysis of all vital organs was done with haematoxylin and eosin staining to assess the anatomical damage involved. *Results:* Findings of the study revealed that the administration of homeopathic drugs at single bolus dose in acute toxicity study there were no mortality or any signs of toxicity observed after oral administration of drugs up to the dose level that of 2000 µl in rats. So, the LD50 was found to be greater than 2000 ul/100g body weight. Sub-acute toxicity study was conducted for a period of 28 days and we noted that the there were no pathological or biochemical alterations found. *Conclusion:* Study inferred that homeopathic drugs are safe on acute and sub-acute administration for 14 and 28 days respectively. The safety profile is established in experimental animals and further data can be corroborated in humans.

Keywords: Toxicity; Homeopathic drugs; Histopathology; Mortality; Biochemical.

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Introduction

A new system of therapeutics is based on the idea that substances simulating the symptoms of illness could aid the natural healing process of body. Homeopathy involves the use of highly diluted preparations with similar therapeutic effect retained. Homeopaths have claimed that their method of preparing dilutions imparts qualities that retain the therapeutic effect.[1] Homeopathic medicinal products are used in other therapeutic approaches with a different methodological status, such as homotoxicology, isotherapy. Homeopathic medicines represent a significant part of medical economies and despite the growing use of homeopathic medicines worldwide, safety remains a major concern.[2] It is usually taken lightly on safety aspects of homeopathic medicines, as these are often highly diluted when administered. Various controversies dogged homeopathy

primarily that the high dilution of substance could reduce the likelihood of adverse effects.[2] There is lack of scientific reports available on safety data of homeopathic medicines. The various homeopathic medicines such as causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja are available in different formulations or preparations for the treatment of different ailments. These aforementioned selected homeopathic drugs have been studied in depth for the presence/or absence of any toxic substance.

Homeopathic causticum is a popular remedy made from the compound called potassium hydrate. As a homeopathic remedy, causticum has many potential health benefits. It is a key remedy for joint and arthritic conditions and skin problems like severe burns. Homeopaths also prescribe it for tremors and paralysis, urinary disorders, respiratory disorders, hemorrhoids, fibromyalgia and more.[3] Calcarea carbonica is obtained from the calcium in oyster shell. It is commonly used for backaches, joint pain, bone pain, bone growths, arthritis, rheumatoid arthritis pain, carpal tunnel syndrome or even bunions.[4] Medorrhinum is an effective homeopathic remedy produced from the bacterium *Neisseria gonorrhoeae*. The collection of bacterium was done from the urethral samples of a gonorrhoea male patient. It is persuasive homeopathic remedy to cure different ailments such as inflammatory (pelvic disorders) and in pain (ovarian and menstrual).[5] It is generally prescribed for different health settings of kidney, spines, mucous membranes and for nerves. Mercurius corrosive is ultra diluted homeopathic preparation prepared from chemical reaction involving mercuric chloride. It is potent in alleviating variant ulcers with most common ulcerative colitis and accompanying diarrhoea, bleeding, and pain.[6] Formica rufa is prepared from the red ants and mitigate gout, rheumatoid arthritis, nausea, headache and vertigo.[7] Silicea is also a form of the non-metallic element silicon or silicon dioxide as one of the major elements of the earth's crust and a vital constituent in plant structure. Homeopathic silicea is also used for eye, nose, throat, bone, joint, nerve system, and digestive problems. Silicea is especially useful for arthritis, knee inflammation of the bone, weak wrists, and subcutaneous nodules of hip joints when there is painful swelling.[8] Sulphur is an elementary substance, occurring in nature as a brittle crystalline solid, burning in the air with a blue flame. The reputation of Sulphur as a remedy is perhaps as old as medicine. The mineral Sulphur is found in every cell of the body. It is especially concentrated in the hair, skin and nails. It is an extremely important homeopathic remedy. It is used for conjunctivitis, eczema, colds, digestive disorders, nausea, constipation, hemorrhoids, diarrhea and shortness of breath.[9] Thuja occidentalis, also known as northern white-cedar or eastern arborvitae, it is an evergreen coniferous tree, in the cypress family Cupressaceae, which is native to eastern Canada and much of the north, central and upper Northeastern United States, but widely cultivated as an ornamental plant. Thuja is used for respiratory tract infections such as bronchitis, bacterial skin infections, and cold sores.[10] There are very few studies that have targeted the eventual benefice of these selected homeopathic drugs. The safety is the major concern in usage of these aforesaid homeopathic medicines for different ailments. The current study was devised to investigate the safe aspects of homeopathic drugs in acute and sub-acute toxicity study. In addition, the effect of these homeopathic medicines on vital organs is also taken into account.

Materials and Methods

Experimental Animals

Wistar rats weighing 150–250 g were obtained from the Central Animal Facility of AIIMS, New Delhi. The rats were housed individually under normal laboratory conditions with natural light-dark cycle and controlled temperature (20–25^{0}C) and humidity. The animals were acclimatized to the environment for a week prior to experimentation with free access to water and standard diet for rats. Homeopathic medicines were given orally, using feeding cannula, and were observed for incidence of mortality and sign of intoxication daily. Animals were housed in labelled cages and their fur was tagged with methylene blue for identification. The protocol was approved by the Institutional Animal Ethics Committee (983/IAEC/16). After approval, animals were taken, weighed and distributed randomly into appropriate groups for conducting the study. The experiments were carried out in the premises of the Animal House, Department of Pharmacology, AIIMS.

Chemical and Drugs

All the chemicals used were of analytical grade. The selected homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) were supplied by CCRH, Ministry of AYUSH, Govt. of India, New Delhi.

Dose Calculation of Study Drugs for Experimental Animals

Dose of homeopathic drugs were calculated as per advice of CCRH

Route of administration = per oral

Vehicle for administration = De-ionized water

Standard dose of 2000 µl/100g was considered for acute toxicity study (single bolus dose) and for sub-acute toxicity study, the dose used was ten times less than acute toxicity study and administered daily for 28 days (20 µl/100g body weight).

Experimental design: Toxicity studies

Acute-toxicity study

Evaluation of acute oral toxicity of all homeopathic medicines (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) was carried out according to the OECD guidelines for testing of chemicals-425 (OECD, 2001). Animals were divided into ten groups of homeopathic medicines. A limit test (2000 µl/100g body weight) was performed on female Wistar rats (150–250 g) from the breeding stock. All the animals were observed for behavioural changes and mortality till 14 days after administration of the dose.[11]

Sub-acute toxicity studies

Evaluation of 28-day oral toxicity study of homeopathic medicines was carried out according to the OECD guidelines for testing of chemicals-407 (OECD, 2008). Wistar rats (150–250 g) of both sexes, from our breeding stock were allocated into eleven groups (n = 5/sex/group). Group I received the normal control (1 ml/kg body weight, 1% saline) and group II-XI received Causticum, Calcarea, Medorrhinum Mercurius, Formica, Proteus, Silica, Placebo, Sulphur, Thuja respectively. Drug/vehicle was administered daily, 20 µl/100g orally for 28 days via oral gavage. Behavioural parameters, Body weight and any toxic signs were noted down on daily basis.[12]

Evaluation parameters

Biochemical Parameters: The different biochemical parameters were evaluated with the help of standard analytical kits from Erba Diagnostic Kits (Transasia Biomedical Ltd.) Mumbai, India according to manufacturer instructions. The biochemical analysis measured were triglycerides (TG), high density lipoprotein (HDL), blood glucose (GLU).

Clinical chemistry: Liver enzymes SGOT and SGPT and serum creatinine level were estimated in testing samples using kits from Span Diagnostic Labs, Karnataka, India.

Haematological parameters

The total counts of Red blood cell, white blood cells, platelets, haemoglobin, BT and CT were measured using haematological analyser (Sysmex XS-1000i). The analysed haematological parameters were RBC, WBC, HB, platelets, bleeding and clotting time.

Necropsy: After the experimental period, the rats were euthanized by cervical dislocation and subjected to gross necropsy and the findings were recorded.

Organ weights: After detailed gross necropsy examination, the following vital organs Liver, Kidney, Heart, Brain, testis and ovaries, were collected from each animal and the weights were recorded.

Histopathology: The following organs and tissue samples were collected from all the animals and preserved in 10% buffered neutral formalin. They were sliced adequately wherever necessary. After a minimum of 24 hr fixation, they were sampled and processed by conventional methods, paraffin blocks were made and 6 µm paraffin in sections was stained with haematoxylin and eosin. They were examined under a light microscope for changes in structure and the pictures taken with digital camera attached to the eyepiece of the light microscope. All deviations from normal histology were recorded and compared with corresponding controls- Liver, Kidney, Brain, Heart, testis and Ovaries.[13]

Statistical analysis

All data produced in this study were represented as Mean ± SEM (n=6). ANOVA was used to calculate the comparison between groups followed by Dunnett's multiple comparison tests. The statistical tools were performed by Graphpad Prism version 5.03, San Diego, CA, USA considering $p < 0.05$ to be statistically significant.

Results

Effect of homeopathic drugs on acute oral toxicity study

In acute toxicity study, we observed no significant deviation in behavioural patterns of all the experimental animals for study period of 14 days. There were no mortality or toxic signs

were observed in homeopathic medicines treated groups which indicate that oral LD_{50} was found to be greater than 2000 µl/100g body weight. The numbers of animals alive/tested were represented in Table 1.

Effect of homeopathic drugs on sub-acute oral toxicity study for 28 days

The homeopathic medicines were administered to experimental animals daily for a period of 28 days in accordance to OECD-407. We found that there was no statistical significance difference in behavioural parameters like walking, sleeping and eating pattern in all the experimental animals and we found no unusual activity among any group. We noted down that there was no sign of toxicity in experimental groups.

Body weight

No significant changes in body weights of homeopathic medicines treated rats were observed when compared with normal control group on day 7, 14, 21 and for 28 days. The mean change in body weight of normal control and treated groups in male (Fig. 1) and female (Fig. 2) Wistar rats was monitored and difference found among groups is non-significant statistically.

Relative organ weight

After daily administration of homeopathic medicines for 28 days, we observed that there were no significant changes in mean organ weight of different organs. No signs of Gastric ulceration or erosion were observed. Change in mean organ weight of different organs in control and treated groups (Data not shown).

Effect on haematological parameters

Table 1: Results of Acute Oral Toxicity of Test Compounds

S. No.	Groups	Days									
		1	2	3	4	5	6	7	8	9	10
		No. of Animals Alive/ Tested									
1	Causticum	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
2	Calcerea	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
3	Medorrhinum	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
4	Mercurius	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
5	Formica	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
6	Proteus	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
7	Silica	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
8	Placebo	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
9	Sulphur	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
10	Thuja	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

Table 2: Effect of administering different homeopathic drugs on Hematological parameters in Wistar rats for period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Hematological parameters					
	RBC	WBC	Platelets	Hemoglobin	BT	CT
Normal Control	9.14 ± 0.22	15.86 ± 1.00	966.2 ± 8.29	14.14 ± 0.51	7.07 ± 0.04	1.73 ± 0.03
Causticum	9.02 ± 0.19	16.27 ± 1.41	971.2 ± 11.21	14.60 ± 0.32	7.02 ± 0.06	1.73 ± 0,02
Calcerea	9.06 ± 0.2	14.06 ± 0.95	977.6 ± 5.71	14.14 ± 0.56	7.02 ± 0.03	1.75 ± 0.03
Medorrhinum	8.90 ± 0.11	16.52 ± 1.30	977.0 ± 7.82	14.36 ± 0.56	7.05 ± 0.04	1.77 ± 0.04
Mercurius	9.26 ± 0.17	17.33 ± 1.45	984.6 ± 6.74	14.20 ± 0.5	7.07 ± 0.05	1.78 ± 0.01
Formica	9.32 ± 0.14	18.42 ± 1.19	978.6 ± 9.17	14.66 ± 0.35	7.04 ± 0.04	1.76 ± 0.03
Proteus	9.30 ± 0.18	18.90 ± 0.66	970.0 ± 7.99	14.16 ± 0.47	7.04 ± 0.04	1.77 ± 0.03
Silica	9.22 ± 0.13	18.87 ± 1.89	977.4 ± 10.92	14.00 ± 0.50	7.01 ± 0.05	1.75 ± 0.02
Placebo	9.28 ± 0.12	18.28 ± 1.17	975.0 ± 10.50	14.78 ± 0.58	7.04 ± 0.06	1.76 ± 0.04
Sulphur	9.54 ± 0.09	19.13 ± 1.95	970.0 ± 8.20	14.66 ± 0.29	7.03 ± 0.03	1.72 ± 0.04
Thuja	9.44 ± 0.12	18.06 ± 1.30	976.6 ± 10.03	14.06 ± 0.39	7.05 ± 0.06	1.74 ± 0.04

Haematological parameters (RBC, WBC, Platelets, Haemoglobin, BT, and CT) were analysed after daily administration for 28 days. All the haematological parameters of treatment groups were remained within physiological range and found no significant changes. The effect of haematological parameters of treatment groups in compared to normal group is represented in Table 2, 3. In male Wistar rats, no statistically significant ($p > 0.05$) difference was found between the groups and similar pattern was observed in female Wistar rats.

Biochemical analysis also did not produce any significant changes in biochemical parameters (Blood glucose, Serum creatinine, SGOT, SGPT, TG, HDL) of male (Table 4) and female (Table 5) rats as compared to those in normal control group. All animals survived until the scheduled necropsy and their physical and behavioural examinations did not reveal any treatment-related adverse effects. No statistical difference ($p > 0.05$) between groups in male and female animals was noticed that exhibits absence of toxic effects post homeopathic drugs administration.

Effect on biochemical parameters

Table 3: Effect of administering different homeopathic drugs on Hematological parameters in Wistar rats for period of 28 days (Female). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Hematological parameters					
	RBC (million/mm^3)	WBC (thousand/µL)	Platelets (thousand/µL)	Hemoglobin (g/dl)	BT (min)	CT (min)
N. Control	8.26 ± 0.09	13.46 ± 1.01	37.88 ± 1.20	12.88 ± 0.46	6.58 ± 0.10	1.60 ± 0.03
Causticum	8.00 ± 0.14	13.06 ± 1.11	35.22 ± 1.31	13.70 ± 0.51	6.63 ± 0.10	1.60 ± 0.03
Calcerea	8.22 ± 0.14	14.21 ± 0.45	34.28 ± 1.20	13.30 ± 0.58	6.59 ± 0.10	1.60 ± 0.04
Medorrhinum	7.88 ± 0.13	12.71 ± 0.71	35.39 ± 1.43	13.06 ± 0.53	6.63 ± 0.06	1.61 ± 0.04
Mercurius	8.22 ± 0.15	14.40 ± 1.23	35.65 ± 1.35	13.46 ± 0.76	6.61 ± 0.05	1.63 ± 0.05
Formica	8.12 ± 0.19	13.39 ± 1.07	34.86 ± 1.12	13.36 ± 0.57	6.46 ± 0.09	1.62 ± 0.03
Proteus	8.36 ± 0.18	14.30 ± 0.88	32.43 ± 1.23	13.44 ± 0.59	6.60 ± 0.10	1.60 ± 0.04
Silica	7.94 ± 0.12	13.73 ± 1.12	33.86 ± 1.98	13.22 ± 0.76	6.58 ± 0.09	1.59 ± 0.04
Placebo	8.16 ± 0.20	14.07 ± 0.69	36.73 ± 1.76	13.88 ± 0.46	6.62 ± 0.07	1.61 ± 0.04
Sulphur	8.12 ± 0.21	14.50 ± 0.82	38.91 ± 1.65	13.42 ± 0.59	6.67 ± 0.07	1.58 ± 0.06
Thuja	8.44 ± 0.13	13.96 ± 1.31	37.34 ± 1.55	13.58 ± 0.75	6.59 ± 0.08	1.61 ± 0.05

Table 4: Effect of administering different homeopathic drugs on biochemical parameters (SGOT, SGPT, blood glucose, serum creatinine, triglycerides, HDL) of Wistar rats for period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons.

Drug Treatment	Biochemical parameters					
	Blood Glucose	Serum Creatinine	SGOT	SGPT	TG	HDL
Normal Control	103.8 ± 1.49	0.46 ± 0.06	116.0 ± 1.30	45.60 ± 1.80	56.80 ± 2.63	43.20 ± 1.46
Causticum	104.2 ± 1.93	0.42 ± 0.05	115.4 ± 2.46	45.80 ± 1.28	59.00 ± 1.37	43.40 ± 1.56
Calcerea	1102 ± 2.12	0.44 ± 0.07	117.2 ± 2.70	43.40 ± 1.43	59.40 ± 2.5	44.00 ± 1.30
Medorrhinum	103.6 ± 1.77	0.48 ± 0.07	116.8 ± 1.06	46.00 ± 2.73	58.40 ± 1.5	42.00 ± 1.22
Mercurius	104.0 ± 1.81	0.42 ± 0.03	115.6 ± 2.06	46.40 ± 1.80	57.80 ± 2.13	44.80 ± 1.15
Formica	102.8 ± 1.35	0.46 ± 0.05	116.6 ± 1.60	45.20 ± 2.03	59.80 ± 1.53	44.00 ± 1.15
Proteus	105.0 ± 2.07	0.48 ± 0.07	114.4 ± 1.86	47.00 ± 1.51	59.00 ± 2.60	43.80 ± 1.65
Silica	103.2 ± 1.59	0.48 ± 0.08	118.6 ± 1.03	45.80 ± 1.82	60.60 ± 1.20	43.20 ± 1.39
Placebo	100.4 ± 1.16	0.46 ± 0.05	116.2 ± 1.15	44.60 ± 2.42	60.40 ± 2.15	45.00 ± 1.22
Sulphur	101.0 ± 1.22	0.42 ± 0.05	114.8 ± 2.2	44.00 ± 1.51	59.00 ± 1.64	44.20 ± 1.65
Thuja	101.6 ± 1.88	0.50 ± 0.07	114.2 ± 1.42	47.00 ± 1.58	60.20 ± 1.46	45.60 ± 1.03

Histopathological evaluation

No pathological changes were observed in histological section of vital organs measured i.e heart, kidney, liver, testis, ovaries and brain of homeopathic drugs treated (Figs. 3, 4, 5) rats as compared to normal control animals. Morphological changes did not reveal cell degeneration or inflammatory cell infiltration in any of the tested homeopathic drugs. The histological sections of liver showed regular arrangement of cells and sections of kidney also showed normal architecture of glomerulus. Thereby no changes were observed at cellular level of these vital organs. Pictomicrographs of histology section of kidney, liver, heart, brain, testis (male) and ovaries (female) of different homeopathic drugs was depicted in Figs. 3, 4 and 5.

Table 5: Effect of administering different homeopathic drugs on biochemical parameters (SGOT, SGPT, blood glucose, serum creatinine, triglycerides, HDL) of Wistar rats for period of 28 days (Female). All values are Mean ± SE. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant

Drug Treatment	Biochemical parameters					
	Blood Glucose	Serum Creatinine	SGOT	SGPT	TG	HDL
Normal Control	101.8 ± 1.42	0.40 ± 0.03	124.2 ± 1.28	45.80 ± 1.39	52.80 ± 2.43	41.60 ± 1.20
Causticum	101.2 ± 1.15	0.42 ± 0.05	125.6 ± 1.99	42.40 ± 1.63	56.00 ± 2.55	40.00 ± 1.41
Calcerea	100.6 ± 1.91	0.44 ± 0.05	126.2 ± 2.17	44.20 ± 1.93	56.40 ± 3.07	42.80 ± 1.56
Medorrhinum	101.2 ± 1.56	0.46 ± 0.05	124.6 ± 2.04	45.00 ± 2.09	54.20 ± 2.08	43.80 ± 2.22
Mercurius	100.4 ± 1.50	0.44 ± 0.06	125.6 ± 2.13	46.40 ± 2.11	56.20 ± 2.87	43.00 ± 1.64
Formica	99.80 ± 1.46	0.40 ± 0.05	125.4 ± 1.43	43.20 ± 1.82	50.80 ± 2.88	40.40 ± 1.28
Proteus	100.8 ± 1.15	0.46 ± 0.05	126 ± 1.51	44.40 ± 1.96	55.40 ± 2.42	43.00 ± 1.51
Silica	101.8 ± 1.46	0.44 ± 0.05	126.8 ± 1.49	45.60 ± 1.50	52.80 ± 3.02	43.40 ± 1.36
Placebo	100.6 ± 1.77	0.46 ± 0.04	123.6 ± 2.29	43.40 ± 1.43	52.60 ± 2.61	42.00 ± 1.81
Sulphur	101.0 ± 1.22	0.42 ± 0.05	125 ± 1.34	46.60 ± 1.63	50.20 ± 1.98	42.20 ± 1.39
Thuja	102.0 ± 1.41	0.42 ± 0.03	126.6 ± 1.20	46.00 ± 2.12	54.20 ± 2.39	40.80 ± 1.15

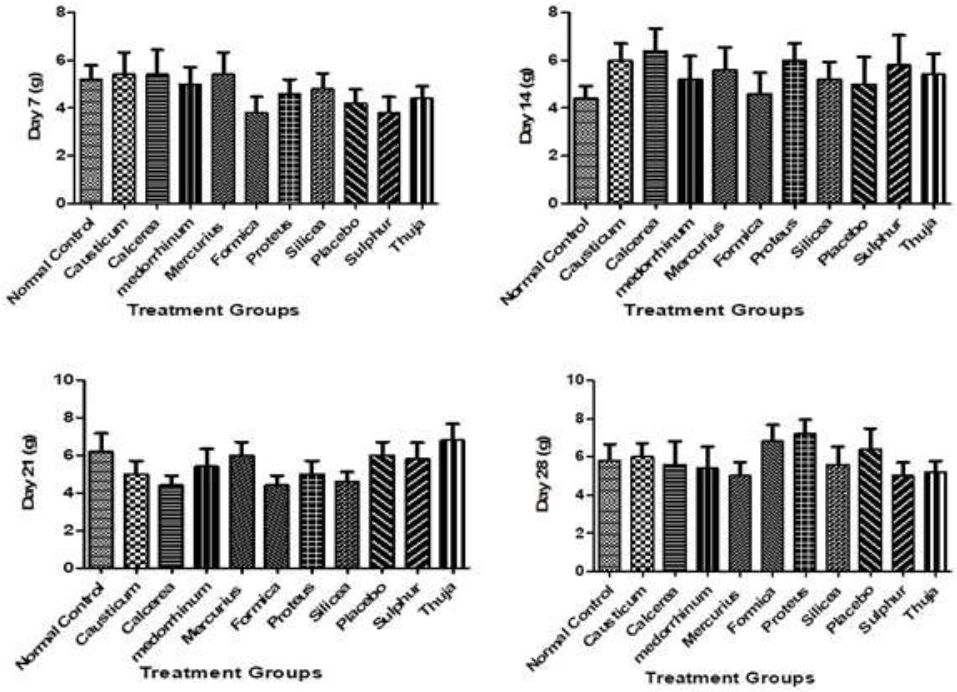

Fig. 1: Effect of administering different homeopathic drugs on mean change in body weight of rat over a period of 28 days (Male). All values are Mean ± SEM. Statistical analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant.

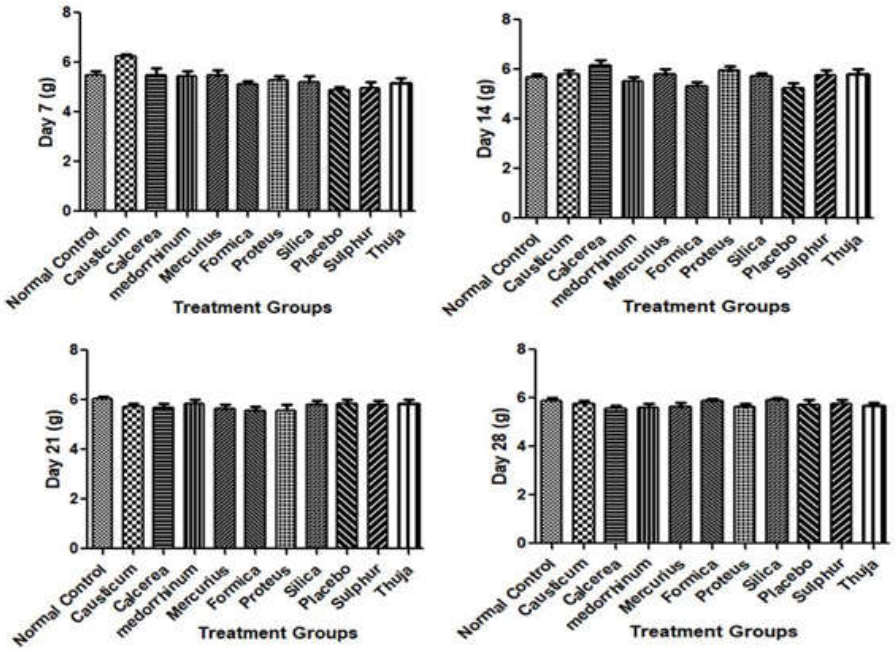

Fig. 2: Effect of administering different homeopathic drugs on body weight of rat on day 7 over a period of 28 days (Female). All values are Mean ± SEM. Statistical mean change in analysis by one-way analysis of variance followed by Dunnett's Multiple Comparisons. $p > 0.05$ represents non-significant.

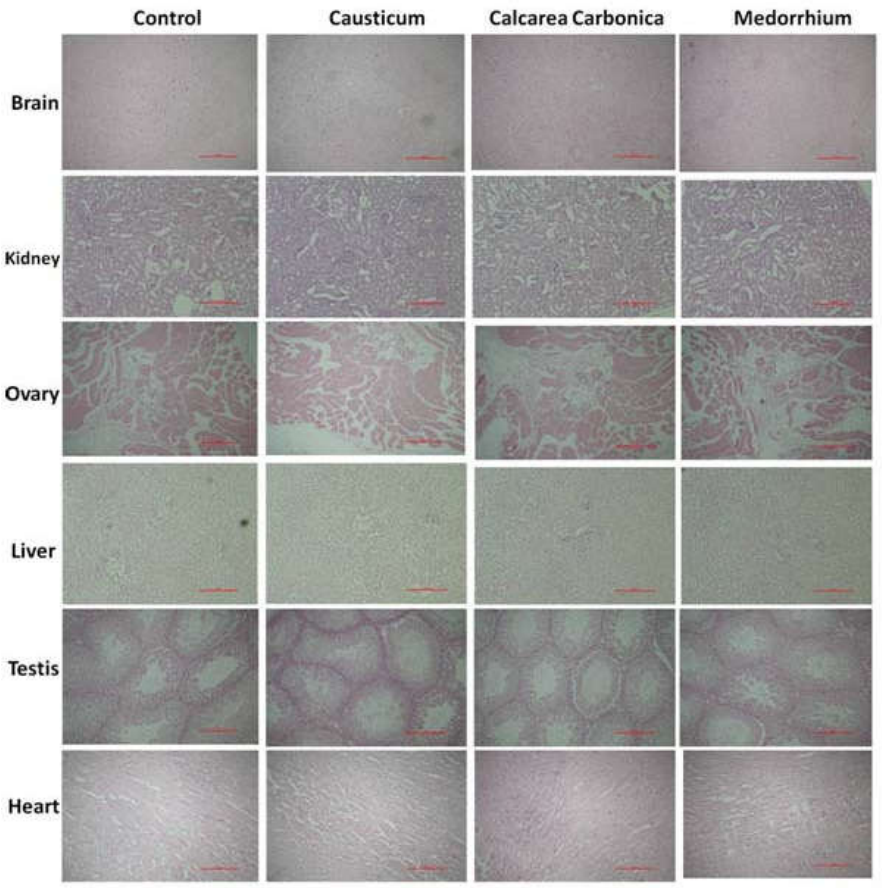

Fig. 3: Effect of normal control and different homeopathic drugs (Causticum, Calcerea carbonica, Medorrhinum) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs

were taken as 10x, scale bar 100 µm.

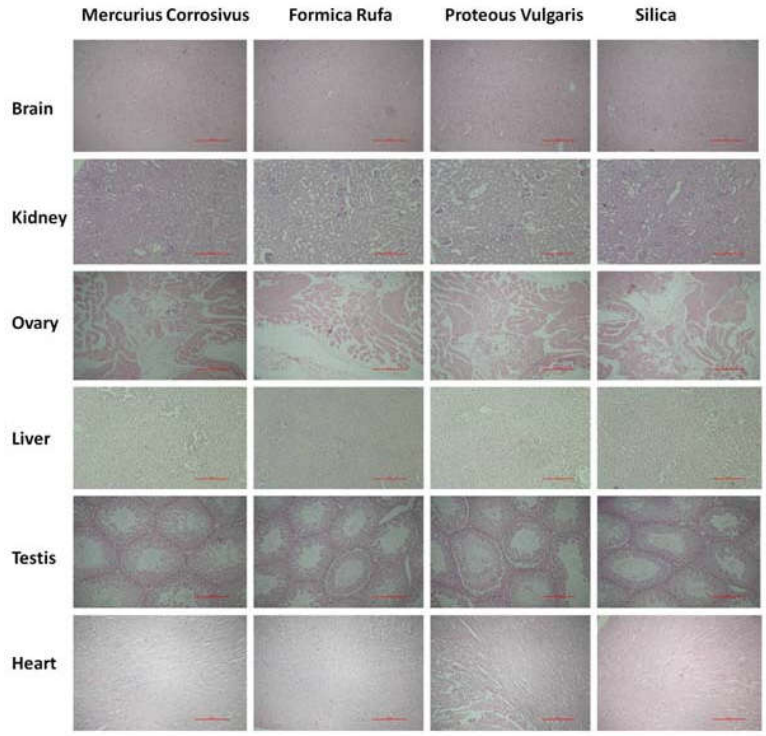

Fig. 4: Effect of different homeopathic drugs (Mercurius Corrosives, Formica Rufa, Proteous Vulgaris, silica) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs were taken as 10x, scale bar 100 µm.

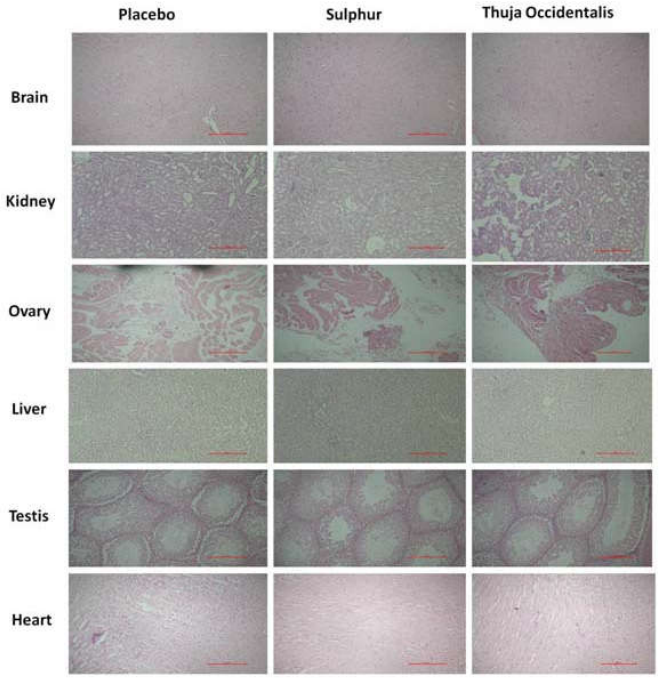

Figure 5: Effect of different homeopathic drugs (Placebo, Sulphur, Thuja Occidentalis) on microscopic examination of different vital organs (Brain, liver, kidney, heart, testis, ovaries) stained by H & E. (Pictographs were randomly taken from group of five rats) Micrographs were taken as 10x, scale bar 100 µm.

Discussion

In the current study, the toxicity profile of selected ultra-diluted preparations was evaluated in accordance to OECD guidelines. As we know that there is a myth in the society that the homeopathic drugs are generally safe. The principle of homeopathic preparations works with the involvement of high dilution factor. However, there is lack of evidences present on safety aspects of homeopathic preparations. Therefore, to scientifically evaluate the toxicity of selected homeopathic drugs was undertaken and experimental studies were carried out.

Acute and sub-acute toxicity study protocol was in corroboration to OECD 425 and 407 respectively.[11,12] In acute toxicity study, the selected homeopathic drugs administered daily up to dose level of 2000 µl/100g body weight (ten times more than effective dose; 20 µl) to the treatment groups and kept in observation for 14 days. We observed the mortality of tested animals for study period and found that the LD50 was found to be greater than 2000 µl/100g body weight in Wistar rats. We examined that up to this dose, mortality in treatment groups was not found and also there were no significant changes in body weights. Previous reports suggest that change in body weight is an imperative marker to study any toxic effects to the individual.[14] Sub-acute toxicity study was carried out for a study period of 28 days to investigate the effect of homeopathic drugs for prolonged period in tested animals. The dose taken for these studies was ten times lower than the dose used in acute toxicity study. Therefore, in our current work, we devised to investigate the effect of homeopathic drugs on body weight, biochemical, haematological and histopathological analysis. The body weight of all the animals were measured at regular time interval i.e. on day 7, 14, 21 and 28. In same manner organ weight of vital organs (liver, kidney, heart, brain, testis and ovaries) were also measured and found to be constant throughout the study period. The findings are in harmony with Ghosh et al. wherein the same pattern of observations was found in organ weight.[15] Meanwhile the behavioural parameters like sleeping, walking and eating were also monitored during the study period and found no abnormality when compared to normal control group.

Sub-acute toxicity study is effective in assessing the effect of homeopathic drugs on target organ, on haematological and biochemical alterations, as these effects are usually not observable in acute toxicity test. Biochemical parameters are the key diagnostic markers to examine any abnormal finding in clinical settings. Biochemical parameters (Blood glucose, Serum creatinine, SGOT, SGPT) were examined in this study. Serum liver function tests provide the information regarding the status of liver. Liver enzymes SGOT, SGPT described the cellular integrity of liver and on the contrary albumin and protein level determine the functionality of liver. As we know that SGOT and SGPT are the key enzymes principally produced by liver cells and any alteration or abnormality to liver, leads to the increase in serum level of these enzymes.[15] Augmentation on these serum enzymes predicts the sign of hepatocellular toxicity, whereas decrease in these leads to the enzyme inhibition. SGPT is the most sensitive marker of liver damage or toxicity. We did not observe any variation in SGPT and SGOT level when compared with normal control animals. Blood glucose is the imperative marker to study the diabetes and prediabetes stage or variation in energy metabolism.[16,17] Blood glucose level showed no significant ($p > 0.05$) changes in treatment groups in comparison to normal control animals. Alteration in hematopoietic is one of the critical steps to determine the drug toxicity. Parameters such as RBC, WBC, Platelets, Hb, BT and CT were studied and found that these were lie within the respective normal range in homeopathic groups which was in comparison to normal control estimates. The gross histology of different organs was performed by haematoxylin and eosin staining. The brain section of Wistar rats in homeopathic drugs showed a symmetric pattern in left and right hemispheres.

The regions of Cerebrum, cerebellum and grey matter revealed healthy Astrocytic cells with prominent nuclei and it is in close proximity to neurons in all tested homeopathic drugs. As stated earlier the microscopic examination of heart section of nine homeopathic drug demonstrated no abnormality in architecture of cardiomyocyte. The normal architecture of cardiac myocytes was maintained with absence of necrosis, vacoulation or infiltration of mononuclear or inflammatory cells. As shown in figures 3,4,5. Histological analysis of liver sections showed a network of hepatocytes that are arranged in single cell thick plates separated by vascular sinusoids. Absence of lipid accumulation, focal inflammation was observed rendering homeopathic drugs safe for hepatic functioning. As depicted in figures 3,4,5. Histology of testis showed well organised germinal epithelium in normal and homeopathic treated groups. No observable difference in seminiferous epithelium of tubules was seemed with single layer of sertoli cells. These depicted no sign of inflammatory

secretions implying no toxicity due to homeopathic drugs on male reproductive organ. Gross histology of ovaries comprises of cuboidal epithelial cells in continuation with peritoneal mesothelium. There was no variation in ovary histology pertaining to homeopathic drug administration. Homeopathic drugs maintain normal homeostasis and thereby did not produce any alteration in microscopic examination. The gross necropsy analysis of various organs did not portray any legends, oedema or observable abnormalities. There we can say that homeopathic drugs (causticum, calcarea, medorrhinum, mercurius, formica, proteus, silica, sulphur, thuja) did not exposed any treatment related adverse effect on various organs.

Conclusion

Acute and sub-acute toxicity study is imperative to assess safety as well as in translation research. LD50 of nine homeopathic drugs used in the study obtained from different sources, was found to be greater than 2000 µl/100g body weight. Selected homeopathic drugs are devoid of toxicity on biochemical, haematological in 28 days repeated sub-acute toxicity study. The findings suggest that homeopathic drugs have NOAEL (no observed adverse effect level) at dose of 20 µl/100g body weight. Further elaborative experimental analysis of chronic toxicity study is imperative to support the findings.

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