Original Article

Safety studies of homoeopathic drugs in acute, sub-acute and chronic toxicity in rats

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Abstract

Background: Homoeopathic drugs are frequently recommended in day to day life as therapeutic agents by homoeopathic practitioners. However, safety of homoeopathic drugs remains a challenge because of the high variability of chemical components involved. **Aim:** The objective of the present study was to investigate the acute, subacute, and chronic oral toxicity of different homoeopathic drugs (*Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) in experimental models. **Materials and Methods:** In acute oral toxicity study, homoeopathic drugs were administered orally at 2000mg/kg body weight, and animals were observed for toxic symptoms till 10 days as per the OECD guidelines. For subacute and chronic toxicity study, homoeopathic drugs were administered for 28 and 180 days, respectively, as per the OECD guidelines. At the end of 28 and 180 days, the animals were sacrificed and toxicity parameters were assessed. Histopathological evaluation of different organs was also performed to assess any toxicity. **Results:** In acute toxicity study, no mortality was found at a dose of 2000 mg/kg which indicates that oral LD_{50} of homoeopathic drugs were more than 2000 mg/kg. The administration of drugs at a dose of 70 mg/kg body weight for 28 and 180 days did not produce any significant change in haematological and biochemical parameters of male and female rats as compared to normal control group. No pathological changes were observed in histology of various organs of treated rats as compared to normal control animals. **Conclusion:** These homoeopathic drugs are safe & produce no toxicity when administered for longer duration.

Keywords: Acute toxicity, *Calcarea phosphoricum* 6X, Chronic toxicity, *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Magnesium phosphoricum* 6X, Subacute toxicity

Introduction

Homoeopathy is practiced in about eighty countries either as independent or as complementary drug to modern system. Homoeopathy is one of the most frequently used complementary and alternative drugs, which uses highly diluted preparations prepared in a specific way unique to Homoeopathy. Homoeopathic preparations have been commonly used for the treatment of various ailments because they are economical, effective, and accessible.[1] About 70%–80% of the world population, particularly in the developing countries, relies on nonconventional drugs in their primary health care as reported by the World Health Organization.[2]

Ferrum phosphoricum is also known as *Ferrum phosphoricum* or *iron phosphate* and is prepared by amalgamating the minerals such as iron and phosphorus. It is formulated by blending three solutions - iron sulfate, sodium acetate, and phosphorus. The resultant product *iron phosphate* is pulverized using large amounts of sugar lactose (also known as milk sugar) to make it nontoxic. *Ferrum phosphoricum* is primarily used for beginning of inflammations. This remedy is most suitable for fevers, rheumatic pain, haemorrhage, nose bleed, headache, sore throat, etc.[3] *Calcarea phosphoricum* is prepared using white calcium phosphate precipitate which is filtered, diluted, dried, and triturated with lactose sugar. Calcium is found naturally within our bodies in our bones and teeth. Within Homoeopathy, *Calcarea phosphoricum* was discovered centuries ago and has been used to treat bone and tooth problems ever since. *Calcarea phosphoricum* is used for the treatment of pain and stiffness in arthritis, neck, and fractures.

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Calcarea phosphoricum works well if the teething process is slow or and the teeth that form are weak and may decay easily. It is also useful in headaches, heartburn, indigestion, and pain after eating. *Magnesium phosphoricum (Magnesium phos)* is found inside the cells of muscles, nerves, bones, brain, and spine. It is a great anti-spasmodic remedy in Homoeopathy. It is useful in cramping of muscles with radiating pain, neuralgic pains, toothache, vertigo, angina pectoris, and soreness of throat.[4] These three medicines are used in lower trituration as boichemic medicines.

It is believed that Homoeopathic formulations are safe without performing any safety study; however, there is no scientific rationale to assume that plants or other medicinal sources, their parts, or derived products, including those of long-standing popular use, are intrinsically safe or beneficial,[5] and it has been reported that many of the drugs used in Homoeopathy with approved pharmacological activity have been rejected as their safety profile is not evaluated, so they would require fewer and simpler preclinical and clinical studies for validation.[6] A scientifically carried out screening is, therefore, important to ascertain safety and efficacy of products used traditionally in Homoeopathy.

Therefore, the present study was carried out to evaluate the safety profile of these homoeopathic medicines i.e. as *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, *Magnesium phosphoricum* 6X which are used for treating various disorders.[7]

Materials and Methods

Animals

The study was carried out in the Department of Pharmacology with the approval of the Institutional Animal Ethics Committee, All India Institute of Medical Sciences (AIIMS), New Delhi (698/IAEC/12). Adult Wistar albino rats (150–200 g) from the Central Animal Facility, AIIMS, were used in the study. Animals were housed under standard laboratory conditions at 25 ± 20°C, and humidity levels were in the range of 30%–70% in groups with free access to food and water *ad libitum*. They were acclimatized to the laboratory conditions for a period of 5 days before the study.

Drugs and chemicals

The homoeopathic drugs in the form of tablets were provided by the Central Council for Research in Homoeopathy, Ministry of AYUSH, New Delhi (manufactured by Kerala State Homoeopathic Corporate Pharmacy Limited, Kerala, India).

Acute toxicity study

Acute oral toxicity test was performed as per the OECD-425 guidelines (OECD, 2001). All the animals were randomly distributed into different treatment groups. Following the fasting period, the female Wistar rats ($n = 5$) were weighed, and the dose was calculated in reference to the body weight. For the main test, a single dose of 2000 mg/kg of each drug was administered to rats in the treatment groups by oral route on day 1. Groups 1, 2, 3, and 4 were given *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X administered orally on day 1 and observed till 10 days, respectively. Food was provided to the rats approximately an hour after treatment. The animals were observed 30 min after dosing, followed by hourly observation for 8 h till 10 days.[8]

Subacute toxicity study

Evaluation of 28-day oral toxicity of homoeopathic drugs was carried out in 5 male and 5 female Wistar rats in each group according to the OECD guidelines for testing of chemicals - 407 (OECD, 2008). Group 1 was given normal saline and served as normal control; Groups 2, 3, 4, and 5 were given *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X administered orally for 28 days, respectively. Body weight were recorded on days 0, 7, 14, 21, 28 and statistical analysis was carried out by one way ANOVA as compared to normal control. At the end of 28 days (subacute toxicity), rats were sacrificed by cervical dislocation. Blood samples were collected for haematological and biochemical analysis under diethyl ether anesthesia through the retro-orbital sinus. The liver, kidney, heart, brain, and ovaries also harvested immediately and organ weights measured. These organs were fixed in 10% formalin for histopathological examination.[9] Biochemical and haematological parameters were measured and analysis of all treatment groups (*Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, *Magnesium phosphoricum* 6X) was carried out as compared to normal control.

Chronic toxicity study

Chronic toxicity tests determine toxicity from exposure for a substantial portion of a subject's life. They are similar to the subchronic tests except that they extend over a longer period of time (180 days) according to the OECD guidelines for testing of chemicals - 452 (OECD, 2008) Chronic toxocity study was conducted on in 5 male and 5 female Wistar rats in each grooup. Group 1 was given normal saline and served as normal control; Groups 2, 3, 4, and 5 were given *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X administered orally for 180 days, respectively. The animals were observed daily for clinical signs of toxicity. The body weight, biochemical and hematological parameters were measured and analysis of all treatment groups (*Ferrum phosphoricum 3X, Ferrum phosphoricum 6X, Calcarea phosphoricum 6X, Magnesium phosphoricum 6X*) was carried out as compared to normal control.[10] A flowchart has been added describing the detailed experimental protocol for all the treatment groups in acute, subacute, and chronic toxicity studies [Figure 1].

Results

Acute oral toxicity study

Acute oral toxicity of the homoeopathic drugs was determined using the limit test at 2000 mg/kg. All animals were observed for mortality for 10 days. All animals survived for 10 days as

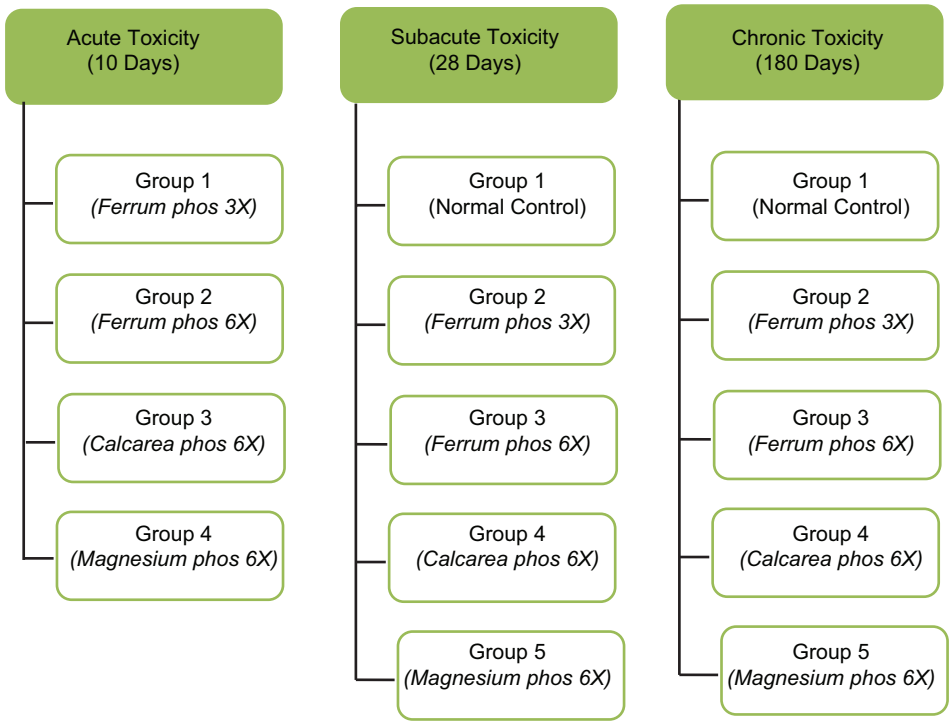

Figure 1: Flowchart describing the detailed experimental protocol for acute, subacute, and chronic oral toxicity studies

Table 1: Effect of administering different homoeopathic drugs on acute oral toxicity of Homeopathic drugs

Groups	Days									
	1	2	3	4	5	6	7	8	9	10
	Number of animals alive/tested									
Ferrum phosphoricum 3X	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Ferrum phosphoricum 6X	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Calcarea phosphoricum 6X	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
Magnesium phosphoricum 6X	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5

shown in Table 1, indicating that 2000 mg/kg of homoeopathic drugs was considered safe in rats.

Subacute oral toxicity study (28 days)

Effect of administering different homoeopathic drugs on Wistar rats for 28 days

All the animals survived on regular administration of Homoeopathic drugs for 28 days. The results indicated that no significant changes were observed in body weight [Table 2], relative organ weight [Table 3], biochemical parameters [Table 4], and haematological parameters [Table 5] as compared to control. No histopathological changes were observed in the tested animals as compared to normal control.

Effect on body weight change

No significant changes in body weights of treated rats were observed when compared to untreated control groups. The body weight changes of control and treated groups are shown in Table 2.

Effect on relative organ weight

After administration of homoeopathic drugs for 28 days, there were no significant changes observed in relative organ weight of different organs. Treatment with homoeopathic drugs do not cause any change in organ weight. No signs of gastric ulceration or erosion were observed. Relative organ weight of different organs in control and treated groups is presented in Table 3.

Effect on biochemical parameters

After administration of homoeopathic drugs for 28 days, there were no significant changes observed in biochemical parameters (hepatic transaminase level, triglycerides level, high-density lipoprotein level, and serum creatinine level) as compared to control as shown in Table 4.

Effect on haematological parameters

After administration of homoeopathic drugs for 28 days, there were no significant changes observed in haematological parameters (haemoglobin [Hb], red blood cell [RBC], white blood cell [WBC], platelets, blood glucose, and clotting and bleeding time) as compared to normal control animals. The effect of Homoeopathic drugs on the haematological parameters is presented in Table 5.

Table 2: Effect of administering different Homoeopathic drugs on body weight of rat over a period of 28 days

Drug treatment	Change in body weight (g)			
	7th day	14th day	21st day	28th day
Normal control	8.66±0.49	17.67±0.88	29.17±0.54	37.50±0.42
Ferrum phosphoricum 3X	9.33±0.42	18.67±0.98	31.33±0.33	37.50±0.42
Ferrum phosphoricum 6X	8.33±0.33	17.83±0.90	29.17±0.79	38.17±0.40
Magnesium phosphoricum 6X	9.66±0.55	20.83±0.83	29.00±0.73	38.33±0.33
Calcarea phosphoricum 6X	9.66±0.49	20.50±0.88	29.17±0.47	38.33±0.61

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons.
SEM: Standard error of mean

Histopathological evaluation

No histopathological changes were observed in kidney, liver, brain, testis, ovary, and heart of various treated animals in comparison to normal control animals after administration of homoeopathic drugs for 28 days.

Chronic toxicity study (180 days)

Effect on body weight change

No significant changes in body weights of treated rats were observed when compared to untreated control groups. The body weight changes of control and treated groups are shown in Table 6.

Effect on relative organ weight

After administration of homoeopathic drugs for 180 days, there were no significant changes observed in relative organ weight of different organs. Treatment with homoeopathic drugs does not cause any change in organ weight. No signs of gastric ulceration or erosion were observed. Relative organ weight of different organs in normal control and treated groups is presented in Table 7.

Effect on biochemical parameters

After administration of Homeopathic drugs for 180 days, there were no significant changes observed in biochemical parameters (hepatic transaminase level, triglycerides level, high-density lipoprotein level, and serum creatinine level) as compared to normal control as shown in Table 8.

Table 3: Effect of administering different homoeopathic drugs on relative organ weight of Wistar rats on 28th day

Drug treatment	Relative organ weight					
	Liver	Kidneys	Heart	Brain	Testis	Ovaries
Normal control	3.51±0.06	0.72±0.01	0.35±0.01	1.97±0.03	1.08±0.02	0.068±0.002
Ferrum phosphoricum 3X	3.30±0.05	0.72±0.02	0.33±0.01	1.93±0.03	1.05±0.02	0.078±0.001
Ferrum phosphoricum 6X	3.33±0.04	0.73±0.01	0.34±0.01	1.97±0.01	1.06±0.01	0.077±0.002
Magnesium phosphoricum 6X	3.46±0.02	0.72±0.01	0.35±0.01	1.92±0.01	1.03±0.02	0.073±0.001
Calcarea phosphoricum 6X	3.33±0.12	0.72±0.01	0.37±0.04	1.97±0.03	1.05±0.02	0.075±0.001

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean

Table 4: Effect of administering different homoeopathic drugs on biochemical parameters (serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase, blood glucose, serum creatinine, triglycerides, and high-density lipoprotein) of Wistar rats on 28th day

Drug treatment	Biochemical parameters					
	Blood glucose (mg/dl)	Serum creatinine (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	TG (mg/dl)	HDL (mg/dl)
Normal control	97.50±3.19	0.65±0.07	106.4±4.20	42.08±1.62	56.33±2.06	32.06±0.83
Ferrum phosphoricum 3X	102.5±3.34	0.68±0.06	114.9±3.81	44.43±2.13	56.51±2.23	34.47±1.35
Ferrum phosphoricum 6X	101.8±3.16	0.65±0.07	111.1±3.80	43.63±1.67	55.74±2.90	36.74±1.36
Magnesium phosphoricum 6X	101.3±3.97	0.71±0.09	111±4.26	45.78±1.46	55.26±4.03	35.30±1.74
Calcarea phosphoricum 6X	103±3.67	0.66±0.06	107.1±4.72	44.41±1.91	56.65±2.71	37.53±1.33

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate pyruvate transaminase; HDL: High-density lipoprotein; TG: Triglyceride

Effect on haematological parameters

After administration of homoeopathic drugs for 180 days, there were no significant changes observed in haematological parameters (Hb, RBC, WBC, platelets, blood glucose, and clotting and bleeding time) as compared to normal control. The effect of Homoeopathic drugs on the haematological parameters is presented in Table 9.

Histopathological evaluation

No histopathological changes were observed in kidney, liver, brain, testis, ovary, and heart of various treated animals in comparison to normal control animals after administration of homoeopathic drugs for 180 days as shown in Figures 2 and 3.

Discussion

In the present study, the safety profile of various homoeopathic drugs, that is, *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X was evaluated. Homoeopathic drugs were used in day to day life and considered safe. However, there are very limited data available for the safety evaluation of these homoeopathic

Table 5: Effect of administering different Homoeopathic drugs on hematological parameters of Wistar rats on 28th day

Drug treatment	Hematological parameters					
	RBC ($\times 10^6$ cells/mm^3)	WBC ($\times 10^3$ cells/mm^3)	Platelets ($\times 10^3$/cm)	Hemoglobin (g/dl)	Bleeding time (s)	Clotting time (s)
Normal control	731±16.74	16,600±236.6	35.22±1.31	12.25±0.46	829.2±4.85	233.8±2.37
Ferrum phosphoricum 3X	734±18.70	16,683±230.1	34.28±1.20	12.57±0.33	856.7±5.98	239.5±3.19
Ferrum phosphoricum 6X	735.5±23.76	16,933±274.1	35.39±1.43	12.62±0.39	825.2±8.19	238.5±4.02
Magnesium phosphoricum 6X	758.8±17.73	16,442±346.5	35.65±1.35	12.37±0.35	843.3±9.97	242.5±4.34
Calcarea phosphoricum 6X	745.2±18.96	16,650±351.0	34.86±1.12	12.45±0.43	857±4.94	241.5±2.99

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean; RBC: Red blood cell; WBC: White blood cell

Table 6: Effect of administering different Homoeopathic drugs on body weight of rat over a period of 180 days

Drug treatment	Change in body weight (g)					
	30 days	60 days	90 days	120 days	150 days	180 days
Normal control	36.50±0.76	71.50±1.40	104.2±1.70	141.2±1.49	172.5±1.47	205.7±2.17
Ferrum phosphoricum 3X	35.67±1.02	72.00±1.86	101.2±1.42	142.8±1.07	173.5±1.99	204.0±1.41
Ferrum phosphoricum 6X	36.33±0.71	71.33±2.06	103.2±1.93	144.8±1.10	174.0±1.96	203.7±1.92
Magnesium phosphoricum 6X	36.67±0.33	71.17±1.77	101.8±1.62	142.2±1.70	174.0±1.93	206.0±2.55
Calcarea phosphoricum 6X	36.17±1.16	72.00±1.63	102.2±1.88	143.5±1.66	173.0±1.80	204.5±1.97

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean

Table 7: Effect of administering different Homoeopathic drugs on relative organ weight of Wistar rats on 180th day

Drug treatment	Relative organ weight					
	Liver	Kidneys	Heart	Brain	Testis	Ovaries
Normal control	3.83±0.01	0.84±0.03	0.50±0.01	2.04±0.04	1.16±0.01	0.08±0.003
Ferrum phosphoricum 3X	3.80±0.02	0.85±0.008	0.50±0.02	1.99±0.02	1.13±0.01	0.08±0.002
Ferrum phosphoricum 6X	3.73±0.03	0.84±0.01	0.48±0.02	1.99±0.02	1.16±0.01	0.08±0.002
Magnesium phosphoricum 6X	3.72±0.03	0.80±0.03	0.50±0.01	2.03±0.04	1.15±0.01	0.08±0.003
Calcarea phosphoricum 6X	3.78±0.04	0.75±0.01	0.44±0.03	2.03±0.02	1.14±0.007	0.07±0.001

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean

Table 8: Effect of administering different Homoeopathic drugs on biochemical parameters (serum glutamic oxaloacetic transaminase, serum glutamate pyruvate transaminase, blood glucose, serum creatinine, triglycerides, and high-density lipoprotein) of Wistar rats on 180th day

Drug treatment	Biochemical parameters					
	Blood glucose (mg/dl)	Serum creatinine (mg/dl)	SGOT (IU/L)	SGPT (IU/L)	TG (mg/dl)	HDL (mg/dl)
Normal control	100.8±3.25	0.58±0.06	111.8±1.53	47.24±0.87	56.91±2.15	44.89±2.11
Ferrum phosphoricum 3X	104.0±4.17	0.60±0.05	120.4±2.21	49.43±1.40	57.50±2.63	47.81±2.38
Ferrum phosphoricum 6X	105.0±5.36	0.66±0.07	115.6±3.01	51.46±2.42	57.31±3.08	50.07±1.34
Magnesium phosphoricum 6X	104.5±4.71	0.65±0.07	118.0±3.73	49.28±0.68	57.78±2.55	51.30±1.13
Calcarea phosphoricum 6X	105.3±5.33	0.61±0.07	114.8±1.75	48.91±1.97	57.57±2.20	50.53±1.36

All values are Mean±SEM (n=10). Statistical analysis by one-way analysis of variance followed by Dunnett's multiple comparisons. SEM: Standard error of mean; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamate pyruvate transaminase; HDL: High-density lipoprotein; TG: Triglyceride

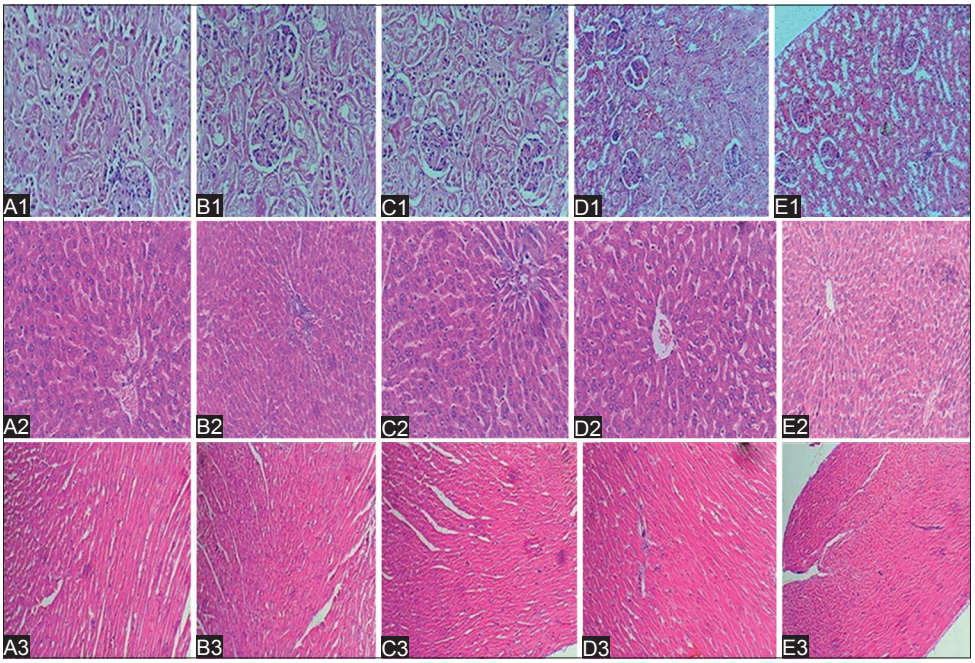

Figure 2: (A1–A3) Normal control (kidney, liver, and heart), (B1–B3) *Ferrum phosphoricum* 3X (kidney, liver, and heart), (C1–C3) *Ferrum phosphoricum* 6X (kidney, liver, and heart), (D1–D3) *Magnesium phosphoricum* 6X (kidney, liver, and heart), and (E1–E3) *Calcarea phosphoricum* 6X (kidney, liver, and heart). No histopathological changes were observed in kidney, liver, and heart tissues of treated group when compared to control group in chronic toxicity study

drugs. Hence, in the present study, it was evaluated that these homoeopathic drugs were safe in acute, subacute, and chronic toxicity studies. These studies were performed according to the OECD guidelines.

The acute toxicity test was conducted according to the OECD guideline 425.[8] The test is a sequential test that uses a maximum of 5 animals. A test dose of 2000, or exceptionally 5000 mg/kg, may be used. The procedures for testing at 2000 and 5000 mg/kg are slightly different. The selection of a sequential test plan increases the statistical power and also has been made to intentionally bias the procedure toward rejection of the limit test for compounds with LD_{50} near the limit dose, that is, to be on the side of safety. As with any limit test protocol, the probability of correctly classifying a compound will decrease as the actual LD_{50} more nearly resembles the limit dose. In the acute toxicity studies, oral LD_{50} of homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) in wistar rats was >2000 mg/kg body weight. The 10-day observation period during the acute oral toxicity study and body weight measurements did not reveal any toxic effect in Wistar rats (data not shown). Necropsy at the end of the study did not reveal any gross pathological abnormality in rats. These observations from oral acute toxicity study suggest

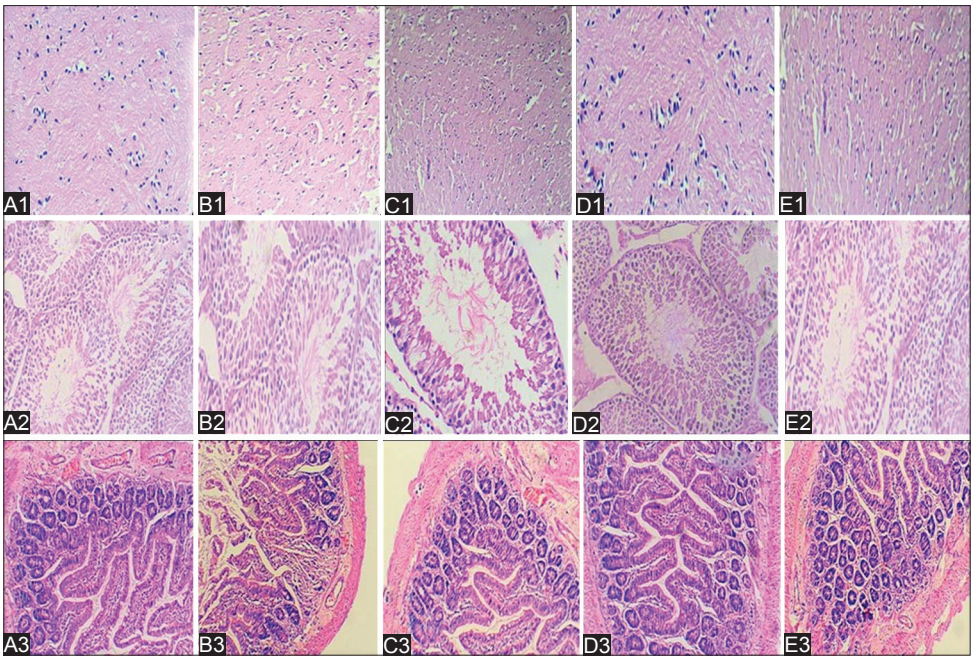

Figure 3: (A1–A3) Normal control (brain, testis, and ovary), (B1–B3) *Ferrum Phosphoricum* 3X (brain, testis, and ovary), (C1–C3) *Ferrum Phosphoricum* 6X (brain, testis, and ovary), (D1–D3) *Magnesium Phosphoricum* 6X (brain, testis, and ovary), and (E1–E3) *Calcarea Phosphoricum* 6X (brain, testis, and ovary). Photomicrographs were taken at magnification (×20). No histopathological changes were observed in brain, testis, and ovary tissues of treated group when compared to control group in chronic toxicity study

that the homoeopathic drugs are practically nontoxic. Hence, there were no mortality or any sign of toxicity observed after oral administration of drugs up to the dose level of 2000 mg/kg in rats. In addition, the LD_{50} was found to be >2000 mg/kg.

The subacute toxicity studies were conducted according to the OECD guidelines 407.[9] The test substance is orally administered daily to several groups of experimental animals, one dose level per group for 28 days. During the period of administration, the animals were observed closely each day for signs of toxicity. Animals that die or are euthanized during the test are necropsied and at the conclusion of the test surviving animals are euthanized and necropsied. A 28-day study provides information on the effects of repeated oral exposure and can indicate the need for further long-term studies. It can also provide information on the selection of concentrations for long-term studies. The data derived from these test guidelines should allow for the characterization of the test substance toxicity, for an indication of the dose-response relationship and the determination of the no observed adverse effect level (NOAEL).

The administration of Homoeopathic drugs such as *Ferrum phosphoricum* 3X, *Ferrum phosphoricum* 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X for 28 days did not produce any significant change in haematological and biochemical parameters of male and female rats as compared to those in normal control group. All animals survived until the scheduled necropsy and their physical and behavioral examinations did not reveal any treatment-related adverse effect. No pathological changes were observed in histological section of heart, kidney, liver, testis, ovaries, and brain of homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) treated male and female rats as compared to normal control animals. These observations from oral acute and subacute toxicity study suggest that the extract is practically nontoxic, found to be safe, and the NOAEL of the extract was found to be 70 mg/kg/day.

The chronic long-term administration of homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) for 180 days was further explored to confirm the safety of homoeopathic medications. As these homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) are mineral based, so their long-term safety studies are needed and it was done according to the OECD guideline 452.[10] The test substance is administered daily to several groups of experimental animals, normally for 6 months. This duration is chosen to be sufficiently long to allow any effect of cumulative toxicity to become manifest, without the confounding effects of geriatric changes. The test substance is normally administered by the oral route. The study design may also include one or more interim kills, for example, at 3 months, and additional groups of animals may be included to accommodate this. During the period of administration, the animals are observed closely for signs of toxicity. Animals that die or are killed during the test are necropsied, and at the conclusion of the test, surviving animals are killed and necropsied. The body weight and the relative organ weights of experimental animals

did not show any significant change after administration of the different homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) for 180 days when compared to the control group. Comparison of relative organ weights between treated and control group of animals has conventionally been used to evaluate the toxic or adverse effects of test compounds or drugs. Relative organ and change in body weight is also used as an assessment of therapeutic response to drugs. This means that these homoeopathic drugs did not have any adverse effect on experimental animals that would cause them to decrease appetite. This signifies that the organ weights did not indicate any toxic or adverse effect from Homoeopathic drugs.

Haematological parameters analyzed included the complete blood count of experimental and control group animals. Analysis of blood parameters in animal studies is relevant to evaluate the risk of alterations of the haematopoietic system in toxicity studies, for necessary application to humans. In this study, administration of the different homoeopathic drugs after 180 days induces no significant change in all haematological parameters as compared to control group. The effect on Hb concentration and WBC count indicated the unlikelihood of the homoeopathic drugs to induce anemia even after long use. Assay of biochemical parameters was performed to evaluate the liver, renal, lipid, and glycemic profiles of experimental compared to control animals, in order to give insight into pathological changes and nature of disease. In this study, assay of the liver profile parameters (serum glutamic oxaloacetic transaminase, and serum glutamic pyruvic transaminase) revealed normal functioning of the liver after 180 days of administration at different homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X), with normal values in experimental animals as compared to control group. The bleeding time in rats was measured by tail tip amputation method and is usually defined as the time of the first cessation of bleeding. No significant changes were observed in bleeding time of Homoeopathic drugs as compared to normal control animals and the data obtained is in correlation to the previous studies.[5,11] Creatinine is indicator of glomerular filtration rate, which is an indicator of the renal function. The renal profile parameters (creatinine and blood urea nitrogen) are in normal range in all experimental animals for 180 days, when compared to control group. Histopathological examinations of the liver, kidney, heart, brain, testis, and ovaries did not reveal any morphological change after administration of the homoeopathic test compounds.

Thus, we corroborate that the mineral-based homoeopathic drugs (*Ferrum phosphoricum* 3X and 6X, *Calcarea phosphoricum* 6X, and *Magnesium phosphoricum* 6X) are safe and produce no toxicity even administered for longer durations. These can be invariably used for clinical purposes. Further, this study can be elaborated and effect of homoeopathic drugs on DNA damage and genotoxicity can further be evaluated to study the scope of these drugs on molecular levels.

Conclusion

These homoeopathic drugs are considered safe as no adverse effect on biochemical and hematological parameters and histopathology of heart, kidney, liver, brain, ovaries, and testis was observed even after administering these drugs for 180 days.

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Conflict of interest

None declared.

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Sicherheitsstudien Von homöopathischer Arzneimittel bei akuter, subakuter und chronischer Toxizitat bei Ratten

Auszug

Hintergrund: Homöopathische Arzneimittel werden im täglichen Leben häufig als therapeutische Mittel durch homöopathischen Praktikern empfohlen. Allerdings bleirt die Sicherheit von homöopathischen Arzneimitteln aufgrund der hohen Variabilität der beteiligten chemischen Komponenten nach meter eine Herausforderung.

Ziel: Die vorliegende Studie untersuchte die akute, subakute und chronische, orale Toxizität verschiedener homöopathischer Medikamente (*Ferrum phos*. D3, *Ferrum phos*. D6, *Calcarea phos*. D6, *Magnesium phos*. D6) in experimentellen Modellen.

Materialien und Methoden: In der akuten oralen Toxizitätsstudie wurden homöopathische Arzneimittel oral in einer Dosierung von 2000 mg/kg body weight verabreicht. Die Tiere wurden für 10 Tage auf toxische Symptome nach OECD-Richtlinien beobachtet. Für die subakute und chronische Toxizitätsstudie wurden homöopathische Arzneimittel für 28 Tage bzw. 180 Tage entsprechend den OECD-Richtlinien verabreicht. Am Ende der 28 und 180 Tage wurden die Tiere getötet und Toxizitätsparameter bestimmt. Histopathologische Bewertungen der verschiedenen Organe wurden durchgeführt, um jede Toxizität zu beurteilen.

Ergebnisse: In der akuten Toxizitätsstudie zeigte sich keine Sterblichkeit bei einer Dosis von 2000 mg/kg, was darauf hinweist, dass orale LD50 von homöopathischen Arzneimitteln höher als 2000 mg/kg ist. Die Verabreichung von Arzneimitteln in einer Dosis von 1000 mg/kg Körpergewicht für 28 und 180 Tage führte zu keiner signifikanten Veränderung der hämatologischen und biochemischen Parameter von männlichen und weiblichen Ratten im Vergleich zur normalen Kontrollgruppe. Es wurden keine pathologischen Veränderungen in der Histologie der verschiedenen Organe der behandelten Ratten im Vergleich zu normalen Kontrolltieren beobachtet.

Fazit: Diese homöopathischen Medikamente sind sicher und produzieren keine Toxizität, wenn sie für längere Dauer verabreicht werden.

Estudio de seguridad oral aguda, subaguda y crónica de los medicamentos homeopáticos en ratas

RESUMEN

Fundamento: Los médicos homeópatas recomiendan frecuentemente los medicamentos homeopáticos en la vida cotidiana como agentes terapéuticos. Sin embargo, la seguridad de los medicamentos homeopáticos sigue constituyendo un reto debido a la elevada variabilidad de los componentes químicos implicados. En el presente estudio se investigó la toxicidad oral aguda, subaguda y crónica de diferentes fármacos homeopáticos (*Ferrum phosphoricum 3X*, *Ferrum phosphoricum 6X*, *Calcarea phophorica 6X*, *Magnesium phosphoricum 6X*) *en modelos experimentales*.

Métodos: En el estudio de toxicidad oral aguda, los medicamentos homeopáticos se administraron por vía oral a dosis de 2.000 mg/kg body weight, observando a los animales en cuanto a síntomas tóxicos hasta 10 días, tal como imponen las directrices de la OCDE. En el estudio de toxicidad subaguda y crónica, los medicamentos homeopáticos fueron administrados durante 28 días y 180 días, respectivamente, conforme indican las directrices de la OCDE. Al final de los 28 y 180 días, se sacrificó a los animales y se evaluaron los parámetros de toxicidad. Asimismo, se efectuaron evaluaciones histopatológicas de diferentes órganos para evaluar cualquier tipo de toxicidad.

Resultados: En el estudio de toxicidad aguda, no se observó mortalidad a una dosis de 2.000mg/kg, lo que indica que la DL50 oral de los fármacos homeopáticos es superior a 2.000mg/kg. La administración de los fármacos a dosis de 1.000 mg/kg de peso corporal durante 28 y 180 días no provocó cambios significativos en los parámetros hematológicos y bioquímicos de las ratas macho y hembra, en comparación con el grupo de control normal. No se observaron cambios patológicos en los resultados histológicos de los diferentes órganos de las ratas tratadas en comparación con los animales de control.

Conclusiones: Estos fármacos homeopáticos son seguros y no producen toxicidad cuando se administran por más tiempo.

चूहों में होम्योपैथिक औषधियों के तीव्र मौखिक, अर्धजीर्ण और जीर्ण सुरक्षा अध्ययन।

सार

पृष्ठभूमि: होम्योपैथिक औषधियों का अक्सर होम्योपैथिक चिकित्सकों द्वारा चिकित्सीय घटकों के रूप मे रोज़मर्रा की ज़िंदगी में परामर्श दिया जाता है, हालांकि, होम्योपैथिक औषधियों की सुरक्षा अभी भी इनमें शामिल रासायनिक घटकों के उच्च परिवर्तनशीलता की वजह से एक चुनौती बनी हुई है। प्रस्तुत अध्ययन में विभिन्न होम्योपैथिक औषधियों (फेरम फॉसफोरस 3X, फेरम फॉसफोरस 6X, कैल्केरिया फॉसफोरम 6X, मैग्नेशियम फॉसफोरस 6X) के प्रयोगात्मक मॉडलों में तीव्र, उप–तीव्र और जीर्ण मौखिक विषाक्तता की जांच की गई।

विधि: तीव्र मॉखिक विषाक्तता अध्ययन में होम्योपैथिक औषधियाँ 2000 मिलीग्राम/किलो की मात्रा में मौखिक रूप से दी गई तथा ओईसीडी के दिशानिर्देशों के अनुसार 10 दिनों तक जानवरों में विषाक्त लक्षणों को देखा गया। उप तीव्र और जीर्ण विषाक्तता अध्ययन के लिए, ओईसीडी के दिशा–निर्देशों के अनुसार होम्योपैथिक औषधियाँ क्रमशः 28 दिनों और 180 दिनों के लिए प्रदान की गई। 28 और 180 दिनों के अंत में, जानवरों का बलिदान किया गया और विषाक्तता के मापदंडों का मूल्यांकन किया गया। विभिन्न अंगों के ऊतकविकृति मूल्यांकन भी, किसी भी प्रकार की विषाक्तता का आकलन करने के लिए किया गया।

परिणाम: तीव्र विषाक्तता अध्ययन में, 2000 मिलीग्राम/किलो की खुराक में कोई मृत्यु दर नहीं पाई गई जो इंगित करता है कि होम्योपैथिक औषधियों के मौखिक एलडी 50, 2000 मिलीग्राम से अधिक नहीं थे। 28 और 180 दिन के लिए प्रदान की गई औषधि की 1000 मिलीग्राम 1 किलो शरीर भार खुराक का सामान्य नियंत्रण समूह की तुलना में नर और मादा चूहों के रक्त संबंधी और जैव–रासायनिक मापदंडों में कोई महत्वपूर्ण परिवर्तन उत्पन्न नहीं हुए। सामान्य नियंत्रण जानवरों की तुलना में उपचारित चूहों के विभिन्न अंगो के ऊतक विज्ञान में कोई रोग संबंधित परिवर्तन नहीं देखे गए।

निष्कर्ष: ये होम्योपैथिक दवाएँ सुरक्षित है और लंबी अवधि के प्रयोग में कोई विषाक्तता उत्पादन नही करती।