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Evaluation of safety profile of homoeopathic mother tinctures

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ABSTRACT

Background: Mother tinctures are commonly prescribed in day to day practice as therapeutic agents by homoeopathic practitioners. However, being the base preparation of medicines, safety of mother tinctures still remains a challenge because of the high variability of chemical components involved.The present study investigated the acute and sub-acute oral toxicity of different homoeopathic mother tinctures *(Bellis perennis, Curcuma longa, Rauwolfia serpentina, Ricinnus communis, Tribulus terrestris and Terminalia arjuna)* in experimental models.

Methods: Toxicity studies were conducted to assess the level to which substances are toxic for humans and animals. In acute oral toxicity study, different homoeopathic mother tinctures were administered orally (a single dose of 4 ml/kg)and animals were observed for toxic symptoms till 14 days as per OECD (Organisation for Economic Co-operation and Development) - 423 guidelines. For sub-acute toxicity study, 28 day oral toxicity of mother tinctures (4 ml/kg daily) was carried out according to the OECD guidelines for testing of chemicals - 407. At the end of 28 days, the animals were sacrificed and toxicity was assessed on parameters such as blood, biochemistry and histopathology.

Results: Results indicate that there were no toxic symptoms observed in tested animals. Results of sub-acute toxicity study did not show any change in body weight, haematological and biochemical parameters as compared to control. The histopathological examination of kidney and liver also did not reveal any organ toxicities.

Keywords: Acute toxicity, Histopathology, Homoeopathic mother tinctures, Sub-acute toxicity

INTRODUCTION

Homoeopathy is one of the most frequently used Complementary and Alternative Medicine (CAM), which uses highly diluted preparations prepared in a specific way unique to Homoeopathy. Plants are major sources of homoeopathic medicines. The medicinal plants are rich in secondary metabolites (which are potential sources of drugs) and essential oils which are of therapeutic importance. The important criteria claimed for homoeopathic medicine in various ailments are that they are economical, effective and accessible.[1] About 70-80% of the world population, particularly in the developing countries, relies on non-conventional medicine in their primary health care as reported by the World Health Organisation.[2] It was believed that homoeopathic products are free from side effects,[3] but it has been reported that many of the plants used in Homoeopathy with approved pharmacological activity have been rejected as their

safety profile is not evaluated[4] or they have toxic effects, e.g. Ricin a constituent from *Ricinus communis* is the most poisonous naturally occurring substance, one seed of which can cause even death.[5] Hence plants can prove to be a major boon to pharmaceuticals, if their safety profiles are properly assessed.

However, safety still remains a challenge because of the high variability of chemical components involved. Various homoeopathic mother tinctures were selected based on the earlier provings of *Ricinus communis, Rauwolfia serpentina, Bellis perennis, Curcuma longa, Terminalia arjuna* and *Tribulus terresteris* conducted by Central Council for Research in Homoeopathy.

The aim of the present study was therefore to evaluate the toxicity of the mother tinctures *viz*. *Ricinus communis, Rauwolfia serpentina, Bellis perennis, Curcuma longa, Terminalia arjuna* and *Tribulus terresteris* so as to develop the preliminary safety profile of the mother tinctures.

MATERIAL 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 (673/IAEC/12). Adult male 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 ± 2^{0}C in groups with free access to food and water *ad libitum*. They were acclimatised to the laboratory conditions for a period of 5 days before the study.

Drugs and chemicals

The homoeopathic drugs in the form of mother tinctures, on the request of Central Council for Research in Homoeopathy, Department of AYUSH, New Delhi were supplied by Dr. Willmar Schwabe India Pvt. Ltd., Noida, India.

Selection of homoeopathic mother tinctures

Homoeopathic mother tinctures that were selected for evaluating the safety profile were *Ricinus communis* (containing 94% v/v alcohol), *Rauwolfia serpentina* (containing 77% v/v alcohol), *Bellis perennis* (containing 65% v/v alcohol), *Curcuma longa* (containing 60% v/v alcohol), *Terminalia arjuna* (containing 82%v/v alcohol) and *Tribulus terresteris* (containing 62% v/v alcohol) were manufactured according to the Homoeopathic Pharmacopeia of India.[6]

Dose calculation

Recommended dose given for rats is 40 µl/100 g body weight (400 µl/kg body weight), per orally with de-ionised water (360 µl) as vehicle for administration.

Acute toxicity Study

Acute oral toxicity test was performed as per OECD (Organisation for Economic Co-operation and Development) -423 guidelines. Twelve groups of male Wistar albino rats (n = 10) were used in this study (test drugs along with their respective control). All the animals were randomly distributed into six control group and six treated groups. Group I received *Bellis perennis* mother tincture (BPMT), Group II received same percentage of alcoholic solution as of mother tincture (65% v/v) which served as control for BPMT, Group III received *Curcuma longa* mother tincture (CLMT), Group IV received same percentage of alcoholic solution as of mother tincture (60% v/v) which served as control for CLMT, Group V received *Rauwolfia serpentina* mother tincture (RSMT), Group VI received same percentage of alcoholic solution as of mother tincture (77% v/v) which served as control for RSMT, Group VII received *Ricinus communis* mother tincture (RCMT), Group VIII received same percentage of alcoholic solution as of mother tincture (94% v/v) which served as control for RCMT, Group IX received *Tribulus terresteris* mother tincture (TTMT), Group X received same percentage of alcoholic solution as of mother tincture (62% v/v) which served as control for TTMT, Group XI received *Terminalia arjuna* mother tincture (TAMT), Group XII received same percentage of alcoholic solution as of mother tincture (82% v/v) which served as control for TAMT. Following the fasting period, the rats were weighed and the dose was calculated in reference to the body weight. For the main test, a single dose of 4 ml/kg body weight of each mother tincture was administered to rats in the treatment groups, whereas the control groups received vehicle (the respective percentage of alcohol) at dose of 4 ml/kg body weight by oral route. 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 14 days.[7]

Sub-acute toxicity study

Evaluation of 28 day oral toxicity of mother tinctures was carried out according to the OECD guidelines for testing of chemicals-407.[8,9] Seven groups of male Wistar albino rats (n = 6) were used in this study (six test drugs and one control). Group I received normal saline (0.1 ml/10g body weight p.o) and served as control. Group II received BPMT), Group III received CLMT, Group IV received RSMT, Group V received RCMT, Group VI received TTMT and Group VII received TAMT. Body weight was recorded on days 1, 7, 14, 21 and 28. At the end of 28 days (sub-acute toxicity), after an overnight fasting, the rats were sacrificed under anaesthesia using diethyl ether. Blood was collected for haematological and biochemical analysis through the retro-orbital sinus. The liver, kidney and heart were harvested immediately and organ weights were measured. The liver and kidney were then fixed in 10% formalin for histopathological examination.

Statistical analysis

Statistical analysis was performed using graph pad Instat software. All values are Mean ± SE. Statistical analysis was performed by one-way analysis of variance followed by Dunnette's Mutliple Comparison.

RESULTS

The objective of the present study was to evaluate the safety profile of homoeopathic mother tinctures in Wistar rats.

Acute oral toxicity study

Oral LD_{50} of mother tinctures in rats was found to be >4ml/kg body weight. Administration of mother tinctures at a dose of 4 ml/kg body weight showed survival of more than 50% of animals in tested groups. Ten percent mortality was found in four groups viz. *Rauwolfia serpentina* mother tincture, *Curcuma longa* control, *Rauwolfia serpentina* control and *Ricinus communis* mother tinctures [Figure 1]. Thus, results demonstrated that the mother tinctures at a dose of 4 ml/kg can be considered safe in rats.

Sub-acute oral toxicity study

All the animals survived on chronic administration of mother tinctures at a dose of 4ml/kg body weight for 28 days. The results indicated that no significant changes were observed in body weight [Table 1], organ weight [Table 2], biochemical parameters [Table 3] and haematological parameters [Table 4] as compared to control. No histopathological changes [Figures 2 and 3] were observed in the tested animals.

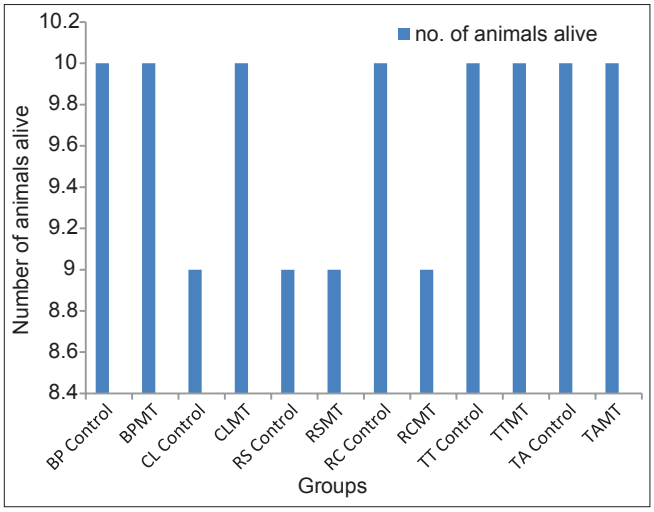

Figure 1: Effect of administering different mother tinctures on acute oral toxicity study for observation period of 14 days

Table 1: Effect of administering different mother tinctures on body weight of rat over a period of 28 days

Drug treatment	Body weight: Change in Body weight (gms)			
	7th Day	14th Day	21st Day	28th Day
Control	10.66±0.33	22.83±1.18	34.33±1.74	46.66±1.62
BPMT	9.83±0.91	20.30±1.36	31.50±1.80	43.52±1.54
CLMT	9.51±0.67	20.66±1.40	33.00±1.36	45.66±1.33
RSMT	10.16±0.88	21.00±1.18	30.83±1.25	42.66±1.43
RCMT	10.50±0.76	21.66±1.52	33.50±1.66	44.33±1.76
TTMT	11.66±0.88	21.83±1.49	31.16±2.15	43.33±2.10
TAMT	12.00±0.85	21.50±1.20	32.83±1.53	43.83±1.72

Values are mean±SEM; n=6 in each group, BPMT: *Bellis perennis* mother tincture; CLMT: *Curcuma longa* mother tincture; RSMT: *Rauwolfia serpentina* mother tincture; RCMT: *Ricinus communis* mother tincture; TTMT: *Tribulus terresteris* mother tincture; TAMT: *Terminalia arjuna* mother tincture

Table 2: Effect of administering different mother tinctures on the organ weight of Wistar rat for period of 28 days

Drug treatment	Organ weight: Change in Organ weight		
	Liver	Kidney	Heart
Control	3.56±0.06	0.72±0.01	0.36±0.007
BPMT	3.29±0.08	0.69±0.01	0.33±0.01
CLMT	3.23±0.15	0.69±0.004	0.32±0.008
RSMT	3.28±0.14	0.69±0.006	0.31±0.01
RCMT	3.05±0.02	0.71±0.008	0.32±0.01
TTMT	3.39±0.08	0.71±0.012	0.32±0.01
TAMT	3.54±0.14	0.69±0.007	0.31±0.01

Values are mean±SEM; n=6 in each group, BPMT: *Bellis perennis* mother tincture; CLMT: *Curcuma longa* mother tincture; RSMT: *Rauwolfia serpentina* mother tincture; RCMT: *Ricinus communis* mother tincture; TTMT: *Tribulus terresteris* mother tincture; TAMT: *Terminalia arjuna* mother tincture

Table 3: Effect of administering different mother tinctures on Biochemical Parameters (Triglycerides, HDL, Serum creatinine, AST, ALT and blood glucose) of Wistar rats for period of 28 days

	Biochemical parameters					
Drug treatment	**Triglycerides level (TG) (mg/dl)**	**HDL value (mg/dl)**	**Serum creatinine level (mg/dl)**	**AST (Aspartate aminotransfrase) (U/L)**	**ALT (Alanine transaminase) (U/L)**	**Blood glucose level (mg/dl)**
Control	56.91±2.16	33.02±0.60	0.99±0.11	97.77±2.73	41.81±1.36	116.66±4.01
BPMT	59.34±2.88	37.77±1.29	1.18±0.11	118.00±2.67	48.52±0.61	99.60±4.48
CLMT	53.69±2.89	36.84±1.1	1.32±0.11	108.99±5.13	47.15±1.35	100.50±2.90
RSMT	55.65±4.93	35.95±1.53	1.35±0.12	111.35±2.91	45.22±2.16	100.50±5.69
RCMT	61.22±4.58	35.00±1.34	1.11±0.18	109.45±2.82	45.1±2.02	103.66±4.20
TTMT	59.82±2.62	32.95±1.7	0.94±0.12	112.07±6.71	44.19±1.50	101.50±4.77
TAMT	55.01±3.30	35.89±1.08	1.16±0.12	105.97±5.10	46.72±1.61	100.83±5.28

Values are mean±SEM; *n*=6 in each group, BPMT: *Bellis perennis* mother tincture; CLMT: *Curcuma longa* mother tincture; RSMT: *Rauwolfia serpentina* mother tincture; RCMT: *Ricinus communis* mother tincture; TTMT: *Tribulus terresteris* mother tincture; TAMT: *Terminalia arjuna* mother tincture; HDL: High density lipoprotein; AST: Aspartate aminotransfrase; ALT: Alanine transaminase

Table 4: Effect of administering different mother tinctures on the blood parameters (RBC, WBC, platelet count, haemoglobin, bleeding time and clotting time) of Wistar rat for period of 28 days

	Haematological parameters					
Drug treatment	Blood parameters					
	RBC (milliom/mm^3)	**WBC (thousand/mm^3)**	**Platelets (lacs/mm^3)**	**Hemoglobin (g/dl)**	**Bleeding time (sec)**	**Clotting time (sec)**
Control	7.78±0.11	17.20±0.17	37.99±0.57	12.53±0.54	834.83±4.23	232.6±3.12
BPMT	7.13±0.22	16.39±0.34	34.81±1.10	11.86±0.31	864.66±5.76	253.4±4.50
CLMT	7.23±0.30	16.89±0.19	35.77±1.47	12.50±0.46	833.66±8.38	245.4±3.75
RSMT	7.22±0.26	16.44±0.19	35.27±1.28	12.42±0.37	859.16±8.20	240.2±3.10
RCMT	7.38±0.28	16.91±0.19	36.07±1.38	12.26±0.65	868.33±11.56	243.5±4.18
TTMT	7.27±0.22	16.52±0.17	35.50±1.11	13.26±0.51	820.16±4.88	238.7±4.20
TAMT	7.43±0.28	17.07±0.22	36.29±1.37	12.90±0.24	836.66±13.94	244.2±3.24

Values are mean±SEM; *n*=6 in each group, BPMT: *Bellis perennis* mother tincture; CLMT: *Curcuma longa* mother tincture; RSMT: *Rauwolfia serpentina* mother tincture; RCMT: *Ricinus communis* mother tincture; TTMT: *Tribulus terresteris* mother tincture; TAMT: *Terminalia arjuna* mother tincture; RBC: Red blood cell; WBC: White blood cell

DISCUSSION AND CONCLUSION

In this study, the safety profile of six homoeopathic mother tinctures viz. *Ricinus communis* (RCMT), *Rauwolfia serpentina* (RSMT), *Bellis perennis* (BPMT), *Curcuma longa* (CLMT), *Terminalia arjuna* (TAMT) *and Tribulus terresteris* (TTMT) were investigated. The major findings of study suggest that these mother tinctures are safe at a dose of 4 ml/kg as no significant changes were observed on biochemical, haematological and histological parameters even for long term administration (28 days toxicity study).

Homoeopathic mother tinctures are used throughout in developed and developing countries and represent a substanstial proportion of the global drug market. Homoeopathic mother tinctures are often mistakenly regarded as safe because they are "natural." Nevertheless, those homoeopathic products also contain some bioactive principles with potential to cause adverse effects, so validation of safety profile of these homoeopathic mother tinctures is needed. Therefore, the objective of present study was to examine the homoeopathic mother tinctures of *Bellis perennis, Curcuma longa, Rauwolfia serpentina, Ricinus communis, Tribulus terresteris* and *Terminalia arjuna* for their safety profile using acute and sub-acute oral toxicity studies.

Acute toxicity study was performed according to OECD guidelines, which gives the range of doses that could be toxic to the animals. It can be used to estimate the therapeutic index (LD_{50}/ED_{50}) of mother tinctures. These mother tinctures were well tolerated and there were no observed adverse effects at a dose of 4 ml/kg. The mortality found in acute oral toxicity study may be due to high alcohol content present in mother tinctures.

In sub-acute toxicity study, mother tincture treated groups did not show any significant changes in body weight increment as compared to control, indicating

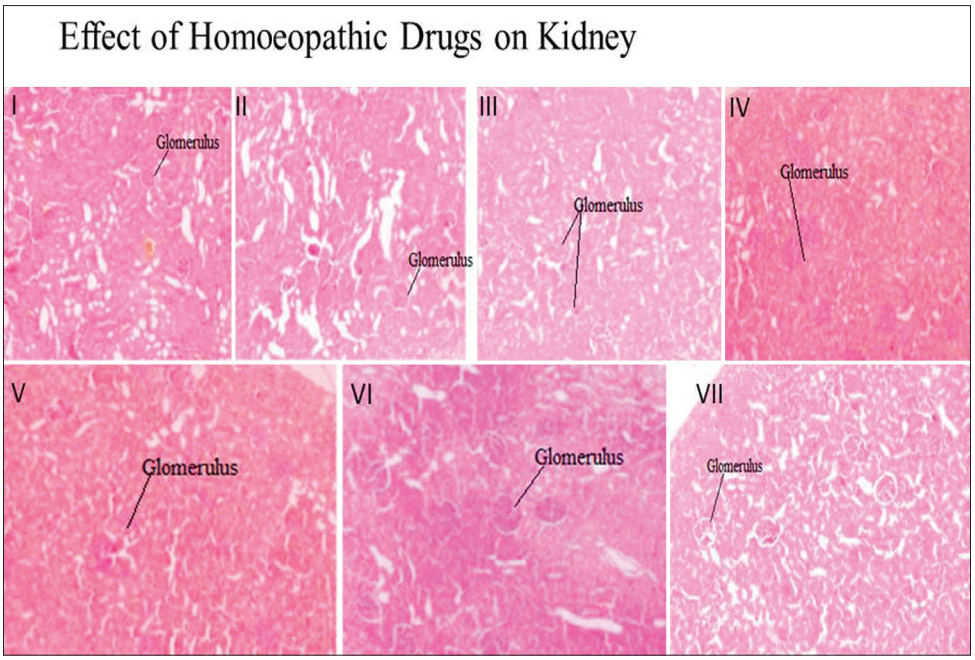

Figure 2: Photomicrographs were taken at magnification (10X). Pictomicrographs demonstrate no degeneration of the glomerular structure or interstitium in any of the groups. Thus, no histopathological changes were observed in kidney tissues of treated group when compared with control group. Group I: Control Group, Group II: BPMT, Group III: CLMT, Group IV: RSMT, Group V: RCMT, Group VI: TTMT, Group VII: TAMT BPMT: *Bellis perennis* mother tincture, CLMT: *Curcuma longa* mother tincture, RSMT: *Rauwolfia serpentina* mother tincture, RCMT: *Ricinus communis* mother tincture, TTMT: *Tribulus terresteris* mother tincture, TAMT: *Terminalia arjuna* mother tincture

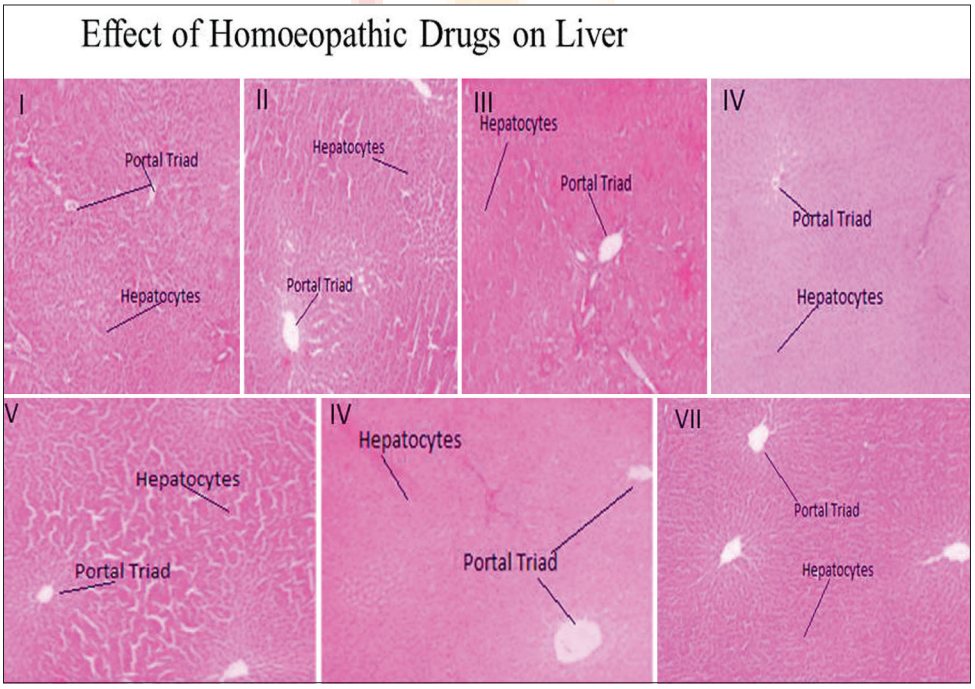

Figure 3: Photomicrographs were taken at magnification (10X). Pictomicrographs demonstrate well-organised lobular structure, normal hepatic cells with clear nucleus, well preserved central vein and portal triad in all the treated groups. Thus, appears normal with no changes in liver tissues of treated groups as compared to control. Group I: Control Group, Group II: BPMT, Group III: CLMT, Group IV: RSMT, Group V: RCMT, Group VI: TTMT, Group VII: TAMT BPMT: *Bellis perennis* mother tincture, CLMT: *Curcuma longa* mother tincture, RSMT: *Rauwolfia serpentina* mother tincture, RCMT: *Ricinus communis* mother tincture, TTMT: *Tribulus terresteris* mother tincture, TAMT: *Terminalia arjuna* mother tincture

that these mother tinctures did not have any adverse effects on body weight. The organ weights and histopathological examination (liver, kidney, heart) of the mother tincture treated groups remained normal. It reveals that these mother tinctures did not produce any toxic symptoms to these vital organs.

There were no significant changes in any liver function parameters such as aspartate aminotransfrase (AST) and alanine transaminase (ALT) levels as compared to control group. The normal level of serum creatinine in all the groups indicate that these mother tinctures did not interfere with renal functioning and renal integrity was maintained. There was no significant difference observed between control and treated group for various hematological parameters such as blood glucose, Hb, red blood cells, white blood cells and platelet count which indicates that these tinctures are not toxic and do not affect circulating red cells.

CONCLUSION

Results demonstrated that all homoeopathic mother tinctures are relatively safe when administered orally to rats. However, further chronic toxicity studies are needed in order to establish the long-term safety of mother tinctures.

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होम्योपैथिक मदर टिंक्चर के सुरक्षात्मक पहलू का मूल्यांकन

पृष्ठभूमि: होम्योपैथी चिकित्सकों द्वारा सामान्यतया मदर टिन्चरों का दिन प्रतिदिन उपचार मे इस्तेमाल किया जाता है। होम्योपैथी में मदर टिन्चर आधार दवा माने जाते है परन्तु रासायनिक विभिन्नताओं के कारण इनकी सुरक्षा अभी तय नहीं है। वर्तमान अध्ययन द्वारा विभिन्न मदर टिन्चरों (बेलिस परनिसिस, कुरकुमा लोंगा, राउवोल्फिया सर्पेन्टिना, रिसीनस कम्यूनिस, ट्राइबुलस टेरेस्ट्रिस एवं टर्मिनेलिया अर्जुना) की तीव्र एवं उपतीव्र मौखिक विषाक्तता की प्रायोगिकी प्रारूपों में जाँच की गई।

विधियाँ: विषाक्तता पर अध्ययन इन पदार्थो के स्तर को जाँचने के लिए किया गया जिस स्तर पर ये मनुष्यों व जानवरों के लिए हानिकारक होते है। तीव्र मौखिक विषाक्तता के अध्ययन में, विभिन्न होम्योपैथिक मदर टिन्चरों की मौखिक खुराक (एक खुराक 4 मिली/किग्रा) दी गई एवं पशुओं में 14 दिन तक ओईसीडी निर्देशिका के आधार पर विषाक्तता लक्षणों का निरीक्षण किया गया। उप तीव्र विषाक्तता अध्ययन में, मदर टिन्चरों की मौखिक खुराक (एक खुराक 4 मिली/किग्रा) दी गई एवं पशुओं में 28 दिन तक ओईसीडी निर्देशिका के आधार पर विषाक्तता लक्षणों का निरीक्षण किया गया। 28 दिनों के बाद पशुओं को मार दिया गया और उनमें रक्त जैव रासायन एवं ऊतकविकृतिविज्ञान के मापदण्डों पर विषाक्तता का अध्ययन किया गया।

परिणाम: परिणामों से इंगित हुआ कि जाँचीय पशुओं में विषाक्तता के कोई भी लक्षण नही पाये गये। उप तीव्र विषाक्तता के अध्ययन परिणामों के भी शरीर भार पर जैव रासायनिक व रक्तीय मापदण्डों में कोई परिवर्तन नही पाया गया। गुर्दे व जिगर के ऊतकविकृतिविज्ञान परीक्षणों में कोई अंगीय विषाक्तता नहीं देखी गयी।