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Designing Toxicological Evaluation of Ayurveda and Siddha Products to Cater to Global Compliance – Current Practical and Regulatory Perspectives

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Abstract:
Despite being age-old and well documented systems of medicine, Ayurveda and Siddha products offers very little actionable data on toxicological profile. The more stringent toxicological requirements of these products as required by prominent regulatory agencies are still unmet. This review documents the toxicological testing of ayurvedic drugs by the globally concurred upon Organization for Economic Co-operation and Development (OECD) protocols for the whole gamut of toxicity studies, and how to adapt these protocols for the requirements of the ayurvedic researcher. This review discusses on viable means to design, conduct and document studies of ayurvedic products for all possible toxicological manifestations in GLP conditions that can withstand global scrutiny and audit. This review explains how to plan the protocol, choose the test systems, chalking out the observation routine, and conduct, acute, sub-acute, sub-chronic, and chronic toxicity studies, with an additional emphasis on carcinogenicity, neurotoxicity, genotoxicity and developmental & reproductive toxicity studies. Determining scientifically consistent toxicological profile of ayurvedic drugs complying with current regulatory practices can be a fillip to their prospects of wide spread acceptance. This article aims at enlightening the researcher on the basics of designing toxicological studies that can cater to cGXP requirements.

Keywords: *Toxicological Testing, Ayurvedic Products, OECD guidelines, Global Compliance*

Introduction:

The ancient medical systems of India dates back to time immemorial and are perhaps one among the few remaining classical medical systems that have vibrantly survived till date. A significant majority of the populace still rely on these traditional sources of medicine for cure and in most instances as an alternative to allopathic pharmacotherapy. But however limited scientific evidence is available to testify the safety and efficacy of these products. The crux of the efficacy data is arrived from the long term clinical experience transmitted and documented among the practitioners [1]. But when it comes to the toxicological profile of these products, only a modicum of actionable data exists. The adoption of more stringent standards for herbal medicinal products by most of the prominent drug regulatory agencies like US Food and Drug Administration (USFDA)[2], Committee on Safety of Medicines, United Kingdom (CSMUK)[3], Medicines and Healthcare products Regulatory Agency (MHRA)[4] and many others have caused a flurry of activity to vouch for the toxicological profile of these drugs.

A globally accepted scientific methodology is therefore required for establishing the toxicological profile of ayurveda and siddha products. A well designed toxicological study should be able to establish the following:

- no-observed adverse effect levels (NOAEL),
- tolerable daily intakes (TDI)
- margins of safety (MOS)

The Organization for Economic Co-operation and Development (OECD) test guidelines [table 1] on toxicity studies in experimental animals represents a harmonized approach in the application of toxicological study protocols and has a global acceptance and credibility.

Rationale for Toxicological Testing of Ayurveda and Siddha Products

The major issue with the Indian Systems of Medicine (ISM) is that still there is only very little scientific evidence to their safety and efficacy; which in part is aggravated by the fact, that it's difficult to evaluate poly herbal medicines using the conventional array of pharmacological and toxicological methods. And thus the proponents theorize on holistic use of plant parts or extracts. The fact to be borne in mind is that these materials consist of hundreds of active ingredients. Many ISM products in use today are based on the

principle of single-chemical isolation from plants or large-scale synthesis. But in many instances, these single chemical entities elicit adverse effects when used alone. Therefore practitioners feel that the active constituents in a plant are rightly balanced within the plant and any possible untoward or toxic effects of one component would be neutralized by the presence of complementary constituents. There are several publications which states on the potential toxicity of the phyto products. Contamination of these products by pesticides, herbicides, naturally occurring toxins, microbes or adulteration by means of synthetic substitutes is a cause for concern. Toxicity manifestations include hepatotoxicity (most prominent - mild elevations of liver enzymes to fulminant liver failure), nephrotoxicity, and neurotoxicity, hematological, mutagenic and cardiovascular toxicities [5] [6]. Hence there is a need for a fundamentally different approach for toxicological studies that need to be adopted for the Ayurvedic and Siddha products. In light of the above stated facts, an integrated approach for safety assessment focused on the hazard identification is imperative. Toxicological protocols prescribed by prominent agencies – USFDA, OECD and WHO rely on *in vivo* tests [table 1]. The type, nature and extent of effect obtained during toxicity studies can help in adequately classifying herbal medicines as non-toxic, moderately toxic or severely toxic on selected biological systems.

Safety of the ISM and its Contentious Products

ISM pharmaceuticals unlike other have the most diverse range of materia – medica. But despite this ISM pharmacotherphy forms as an alternative therapy for chronic illnesses which often cause untoward side effects due to long time exposure of allopathic medication[7]. As interest is being renewed in these traditional systems the benefits of this is hampered by the fear of toxicity some of the categories of these products might elicit especially - *Bhasmas* and its allied products namely *Parpati, Rasayoga* and *Sindoora*.

Accumulated toxicity data on the hazardous effects of heavy metals as propounded by the modern medicine has made the world vary of heavy metals. As a result the beneficial effects produced by heavy metal and other herbo – metallic compounds of ISM are often viewed with suspicion and rightly so. But ISM of medicine has a documented history of safe usage of these medications for the past 2500 years[8], [9]. The metals that are extensively described in Indian and other ancient systems of medicine include gold, silver, arsenic, copper, iron, lead, mercury, and zinc. As far as ISM is concerned, metals have been used mainly as *Bhasma* (ash). Classical texts Ayurvedic texts, *Charaka Samhita* and *Susruta Samhita,* and other medieval works *Astanga Hridaya, Vagbhata* , *Mdhahva Nidan* ,*Sharangadhara Samhita*, and *Bhava Prakash* include ample description of the use of the metals and minerals in the treatment of diseases[10]. They have intricately described the *marana* and *puta* procedures and the different *shodanas* that are prescribed for each preparation[11], the phased spot test developed by the investigators of Central Council for Research in Ayurveda and Siddha (CCRAS) should also be considered. In ISM a great emphasis is placed on *shodhana* (purification) and detoxification of metals and other minerals. The process of *shodhana* is followed by the incorporation of various herbal juices to get the final product. This alters the metallic salt forms and the bioavailability. These processes can also convert the meta various responses, especially the immune responses. It should be borne to thought that metal products are not fist line products in ayurveda.

The therapeutic efficacy of various bhasmas – namely, *swarna bhasma*[12], *tamra bhasma*[13], *abhrak bhasma*[14], *Mandur bhasma*[15], *Muktashukti bhasma*[16], *Yashada bhasma*[17], has been proven in animal models. But however when it comes to the toxic and toxicokinetic effects of these preparations, acute, sub-acute and long term toxicity data is found sorely missing. Plus non-existent data on the heavy metal traces is another conscientious question. It acts as a moot point for nay- sayers to query on the toxic properties of these products. Hence it would be in the best interest of ISM that we test these contentious products under the ambit of

OCED in order to get a wide spread acceptance of the data. Accepting this *de facto* guideline would help infuse credibility to ISM products.

Adapting OECD toxicity protocols to ISM

Whilst applying the OECD toxicity testing protocols to ISM products, we have to take into account several posers that are unique to ISM. The prominent of them being:

(i) Lack of standardized test materials

Unlike testing materials of allopathic and synthetic chemical origin testing ISM products can be challenging. While the former products have very well defined in house standards during the testing phase and pharmacopoeial monographs after acceptance; there are no similar standards when it comes to ISM. However steps in right direction are being taken now by the inclusion of the pharmacopoeial monographs. Still, differences exist between various schools of practitioners. The identity of the herbs, the composition, and the dose differs. Hence it is always better to include a detailed description of the collection, processing, manufacturing process and the formula of the test material in the protocol. As a matter of fact, OECD GLP tenets suggest that the detailed material characterization of the test material should be available before the commencement of the testing[18]. This can be achieved by doing appropriate chemical testing, HPTLC runs for the prominent markers or establishing the identity by more sophisticated methods like gene mapping. Or if its a established product, that is put under scanner the pharmacopoeial monograph of the same can be taken as reference. Especially in case of metallic products while attempting at the toxicological testing of the preparations per se, the standardization of the process is absolutely essential. It's better to develop analytical profiles of these preparations by Gas Chromatography – Mass Spectrometer (GC-MS), X- ray Powder Resonance (XPRD) and deduce the presence of metal traces. This should be the part of the testing protocol.

(ii) Methods of administering test materials (Anupan /Anubhanam)

This is a poser which is unique to ISM products. ISM products by virtue of millennium of tune testing and optimization has found some ingenious methods for mitigating known toxic manifestations of the test materials by co-administering with adjuvants and vehicles like honey, clarified butter, curds etc. These adjuvants and vehicles though not a part of the test material forms a mechanism to reduce the toxicity of the test material. Therefore while testing the ISM test material this issue has to addressed appropriately. This can be done by introducing a vehicle control as one of the test groups and test material with and without vehicle has to be included as other treatment arms. This way, from the experience of the authors, suggests can be an effective way to address this issue. Furthermore, its best to procure these vehicles and adjuvants for the entire duration of the study from a single source to prevent variations.

If there are more than one *anupan* / *anubhanam* that is planned to be tested out, a separate study would be advisable to prevent statistically confounding results. Since the testing involves multiple group wise comparisons, one-way ANOVA followed by Student t-test, all pairs Tukey-Krammer test, Hsu's Multiple Comparion with the Best and Dunnet's test with control group is desirable[19]. Boneferroni's correction can be considered where appropriate [20]. However higher number of animals and extra efforts entailed can be an issue with the ethical committee. Therefore before envisaging these studies, a detailed rationale has to be chalked out in the protocol for the perusal of the team members and ethics committee.

(iii) Polyherbal nature of the test materials

This is another issue that is unique to ISM and alternative therapy testing. While it is going to be a mono-chemical entity or a well characterized poly-chemical entity that is tested in a conventional toxicity testing, testing of ISM products needs a fundamentally different outlook. The OECD and other testing protocols are based on the western science's "reductionist" approach; ISM products at many levels require a "holistic" outlook in terms of understanding test procedures and execution. Typical ISM materials consists of multitude of active principles (ranging from tens to hundreds) its

always a better idea to treat the test material as a "single holistic entity" rather than attempting at a futile reductionist approach. The prior characterization of the test material as listed in the protocol done in house or as evidenced in the supplier's certificate of analysis (COA) should be the key entity on which the testing should be based. As the present day science is yet to come to terms with the enormously complex phyto-pharmaco disposition (especially the phyto pharmacodynamic models) the testing has to concede with the polyherbal nature of the test materials.

(iv) Safety profile and experience of the researcher

The phyto pharmacotheraphy in principle is safe in many instances when compared to its synthetic counterparts. But when testing the toxicity *per se,* the tester should not be biased with the apparent safety of the test materials. Its best to design the protocol to gather complete evidence of non-toxicity as listed in tables 3 and 6. It is better to assemble an experienced research team to understand the implications and interpret the gathered data accurately. The toxicity testing iterations should be tested based on the preliminary toxicity data available and carried on forth. The choice of advanced toxicity testing should be based on the result of acute toxicity at that dose.

An Overview of OECD Testing Protocols Adapted To ISM

Acute Toxicity Studies

These studies are designed to capture the toxic effects elicited by any substance or mixture of substances when administered in single (or rarely multiple) doses to experimental animals over a period not exceeding 24 h. It is useful in preliminary identification of target organs of toxicity and, for revealing delayed effects, if any.

The study also aids in the selection of starting doses for preliminary human studies or dose-ranges for subsequent repeat-dose studies and provides valuable data in cases of acute overdosing in humans. The test compound should be administered (up to the maximum feasible dose) to animals to identify doses causing no adverse effect and doses causing major life-threatening toxicities. Ideally, the acute toxicity studies in animals should be conducted using the same route intended for human administration (oral route is preferred). Refer to table-1, 4.

Newer approach prescribed by OECD:

The Previous methods of acute toxicity testing were based on LD_{50} proposed by Miller and Tainter [21], Litchfield and Wilcoxon [22] and Lorke [23]. These traditional methods for assessing acute toxicity relied on deaths of animals as endpoints, thus giving an LD_{50} value for each substance investigated.LD_{50} is a standard measure of the toxicity of a test substance that will kill half of the sample population of a specific test animal in a specified period through exposure. However newer and better techniques are now set out in the OECD test guidance documents no. 420 (fixed dose procedure) [24], no. 423 (acute toxic class method) [25] and no. 425 (up and down procedure)[26].

Repeat dose studies [27] [28]

Repeat dose studies can extend from 28/90-day to 6-month or even more than a year. Oral toxicity of herbal medicines using repeat doses may be accomplished after initial acute toxicity testing. Long term possible health hazards can be detected by the study. It indicates occurrence of any immunological, neurological or reproductive toxicity of herbs.

Selection of dose levels

Experimental design is critical prior to commencement of such studies. As per regulation at least 3 tests and a control group are necessary. The lowest dose is usually a simple multiple of the therapeutic dose whereas the highest dose is normally chosen to be a nearly toxic dose. However, the highest dose level should not cause a body weight decrease >10–12% relative to concurrent control values and in a dietary study should not exceed 5% of the total diet because of potential nutritional imbalances caused at higher levels or produce severe toxic, pharmacological or physiological effects. The median dose lies somewhere between the two extremes (possibly a geometric mean of the two).The recommended doses are graded exponentially at 0.1%, 1% and 10% of the oral acute

toxicity LD_{50} values. Ideally, the lowest and median dose levels should be selected with a view to demonstrating any dose–response relationships while the lowest dose is expected to cause no observable adverse effects (NOAEL). Refer to table-4.

Preparation and administration of doses

Doses are given 7 days a week by gavage, diet or drinking water for required duration with concurrent monitoring of quantity of herbs involved; to avoid interference with normal nutritional status or cause water imbalance. Refer to table-1

Table 1: Overview of the current OECD Toxicity Testing Protocols and their test Systems

OECD toxicity test guidance #	*Toxicity type*	*Test system*	*Routes*	*Duration*
420[1]/423[2]/425[3]	Acute	Rat (female)	Oral/inhalation	≤ 14 days
407[4]	Sub-acute	Rat	Oral/diet/drinking water	14–28 days
408[5]	Sub-chronic	Rat	Oral/diet/drinking water	30–90 days
452[6]	Chronic	Rat	Oral/diet/drinking water	≥ 6 months
453[7]	Combined chronic and carcinogenicity	Rat/mice/hamster	Oral/dermal/inhalation	18 months to 2.5 years
451[8]	Carcinogenicity	Rat/mouse/hamster	Oral/dermal/inhalation	2 years
473[9]–486[10]	Genotoxicity	Variable	*In vitro/in vivo*	Variable
414[11]/415[12]/416[13]	Developmental and reproductive	Rat, dog	Oral	Variable
424[14]	Neurotoxicity	Rat	Oral/dermal/inhalation	Variable

1. OECD (2001). Test Guideline 420. Acute oral toxicity – fixed dose procedure (FDP). In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
2. OECD (2001). Test Guideline 423. Acute Oral Toxicity – Acute Toxic Class Method. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
3. OECD (2006). Test Guideline 425. Acute Oral Toxicity – Up and Down Procedure (UDP. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris
4. OECD (1995). Test Guideline 407. Repeated Dose 28-day Oral Toxicity Study in Rodents. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
5. OECD (1998). Test Guideline 408. Repeated Dose 90-day Oral Toxicity Study in Non-rodents. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation & Development, Paris.
6. OECD (2008),Test Guideline 452. Chronic Toxicity Studies. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
7. OECD (1981). Test Guideline 453. Combined chronic toxicity/ carcinogenicity studies. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
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9. OECD (1997). Test guideline 473. In Vitro Mammalian Chromosome Aberration Test, In: Draft OECD guidelines for testing of chemicals. . Organisation for Economic Cooperation and Development, Paris
10. OECD (1997) . Test guideline 486. Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in-vivo, In: Draft OECD guidelines for testing of chemicals. . Organisation for Economic Cooperation and Development, Paris
11. OECD (2001) . Test Guideline 414. Prenatal development toxicity study. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
12. OECD (1983) . Test Guideline 415. One-Generation Reproduction Toxicity Study. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris.
13. OECD (2001) . Test Guideline 416.Two-Generation Reproduction Toxicity Study. In: OECD Guidelines for the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris
14. OECD (1997) . Test guideline 424,Neuro toxicity Study, In: Draft OECD guidelines for testing of chemicals. . Organisation for Economic Cooperation and Development, Paris

Table 2:

Test substance characteristics	• Description about the test substance (part of herb or herbo-metallic preparation), • extraction method • type of extract • percentage yield • If possible obtain Certificate of Analysis (COA) eliciting the HPTLC fingerprint of active constituents from the test material supplier or sponsor of the study**.**
Animal selection[1]	• Young, healthy, adult rats of either sex used (age range 8–12 weeks). • OECD recommends nulliparous and non-pregnant female rats for acute toxicity testing. (Female more sensitive picking up subtle effects). • Animals randomized and assigned to the required number of test groups.
Housing conditions[2][3][4]	• animals maintained under standard conditions of humidity (30–70%), temperature (22±3ºC) and 12 h light/darkness cycle • Should be acclimatized to the environment for a week prior to commencement of the studies. • The weight variation in animals or between groups should not exceed 20% of the mean weight. • Food and water provided ad *libitum*. • overnight food starvation is recommended if test substance is orally fed
Test dose preparation and administration	• The maximum volume of freshly prepared herbal medicine that can be administered at once in rodents is 1 mL/100 g of body weight (although can go up to 2 mL/100 g). • For non water vehicles, the toxicological characteristics of the vehicle should be known. . (A test group comprising vehicle control may be considered) • After the substance has been administered, food may be withheld for a further 3–4 h in rats or 1–2 h in mice.

1. NIH (1985). Guide for Care and Use of Laboratory Animals. Publication # 85-23. (1985, revised) DHHS. NIH Publication. Bethesda, Maryland, USA.
2. OECD (1982). Good Laboratory Practice in the Testing of Chemicals. Organisation for Economic Cooperation and Development, Paris, p. 62.
3. WHO (1990). Principles for the Toxicological Assessment of Pesticide Residues in Food, Environmental Health Criteria 104. IPCS/WHO, Geneva
4. NIH (1985). Guide for Care and Use of Laboratory Animals. Publication # 85-23. (1985, revised) DHHS. NIH Publication. Bethesda, Maryland, USA.

Body weight, food and water consumption

Body weight is assessed before commencement of dosing, followed by weekly once during the dosing period and once on the terminal day. Food and water consumption are measured daily or at least weekly. Refer to table-2

Absolute and relative organ weights

At the end of the dosing period, all the animals should be killed humanely and the different organs are carefully isolated and weighed (absolute organ weight).

Relative organ weight (gm) = absolute organ weight (gm) / terminal body weight (gm) X 100.

Gross necropsy and histopathology

Full histopathology should be carried out on all gross lesions and also on the preserved organs and tissues of all animals in the control and high-dose groups. The wet weights of the organs of all surviving animals should be measured soon after dissection. There after preservation in the fixation medium (formol saline) should be carried out. Refer to table- 3

Table 3:

Observations and data monitoring	1. Animals should be observed individually after dosing at least once during the first 30 min, periodically during the first 24 h (with special attention given during the first 4 h), and daily thereafter, for a total of 14 days. 2. Systematic observations are observed for each animal. Gross necropsies should be performed and recorded for each animal including those sacrificed moribund, found dead, or terminated at day 14. 3. It may be necessary to perform histopathological examination of organs showing evidence of gross pathology in animals surviving for 24 h or more after the initial dosing.
Data collection and test reporting	1. Test substance and dosing 2. Test animals 3. Tabulation- body weights (0, 7, 14 days), response-dose level data, time course of onset of signs of toxicity 4. Necropsy and histopathological findings

Toxicokinetic and metabolism data

Herbal medicines may pose difficult problems in toxicokinetic evaluation as the chemical constituents vary in hundreds or thousands (e.g. alkaloids, glycosides, flavonoids, quinines, tannins, polyphenols, and sugars). In any case, if the chemical composition of a plant is well known, then it may suffice to do a toxicokinetic profiling of the major active constituents. Refer to table-4

[1]Data analyses and test reporting [29]

It is similar to reporting of acute toxicity testing, with data on additional toxicity parameters A detailed description of all histopathological findings, Toxicokinetic data if available, Statistical analyses in addition to discussion of results and appropriate conclusions. Refer to table-3

Table 4: Acute toxicity study test methods **

Method	*Principle*	*Procedure*
Fixed dose procedure -420	Observation of clear signs of toxicity at one of a series of fixed-dose levels, thus avoiding death as an endpoint	Groups of animals of a single sex are dosed in a stepwise procedure using the fixed doses of 5, 50, 300 and 2000 mg/kg
Acute toxic class 423	Step-wise procedure based on biometric evaluations, Three female rats are used per step and depending on the mortality and/or the moribund status of the animals, a total of two to four steps may be necessary.	Predefined doses of 5, 50, 300 and 2000 mg/kg body weight are used
Up and down procedure-425	The main test consists of a single, ordered dose progression in which animals are dosed, one at a time, at a minimum of 48-h intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar factor.	Doses from the sequence 1.75, 5.5, 17.5, 55, 175, 550, 1750, 2000 or 5000 mg/kg for specific regulatory needs

** ASTM (1987). E 1163–87, Standard Test Method for Estimating Acute Oral Toxicity in Rats. American Society for Testing and Materials, Philadelphia PA, USA.

Table 5: OECD repeated dose, sub chronic, carcinogenicity, and other toxicity study patterns.

OECD no.	*Name*	*Number of animals*	*sex*	*Duration of study*	*Doses*
407, 408, 409, 410, 412	Repeated dose toxicity study	• At least 10 at each dose level. • 10 in control group. • Additional 10 in top dose group • At least 3 test and a control group	M/F=1 in each group	28-90days or 6month to >1 year	Not >1000mg/kg.bw/day until NOAEL. 0.1%,1%,10% of oral acute toxicity LD50 values
408, 409, 411, 413	Sub chronic toxicity study	• At least 20 at each dose level • 10 in control group • Additional 10 in top dose group • At least 3 test and a control group	M/F=1 in each group	28-90days	Not >1000mg/kg.bw/day until NOAEL. 0.1%,1%,10% of acute oral toxicity LD50 values
451, 453	Carcinogenicity studies and combined chronic toxicity/carcinogenicity studies	• At least 50 animals of each sex at each dose level and a concurrent control group • At least 3 test and a control group	M/F=1 in each group	18-30 months depending on animal species	Based on result of previous sub-chronic toxicity study
414, 415, 416	Developmental and reproductive toxicity studies	• At least 20 at each dose group • 20 in control group • At least 3 test and a control group	All pregnant females at or near parturition	28 days depending on the animal species	>1000mg/kg.bw/day if no observable toxic effects else graduated reduction in dose with NOAEL
424, 471, 473-486	Neurotoxicity and Genotoxicity	variable	variable	variable	variable

M = Male
F= Female
NOAEL = No Observed Adverse Effect Levels

Sub-chronic toxicity studies

As per (WHO) a sub-chronic study is 'having a duration lasting up to 10% of the animal's lifespan, 90 days in rats and mice, or 1 year in dogs'. The main purpose of sub-chronic testing is to identify any target organs and to establish dose levels for chronic exposure studies. The 90-day study provides information on major toxic effects, target organs and the possibility of accumulation, and can provide an estimate of a NOAEL of exposure, which can be used in selecting dose levels for chronic studies and for establishing safety criteria for humans. Toxicological parameters to be monitored are similar to those outlined earlier except that interim hematological and clinical chemistry evaluations may be performed at selected times. Refer to table-5

Combined chronic toxicity and carcinogenicity studies

These long-term observations are defined as studies lasting for the greater part of the lifespan of the test animals, usually 18 months in mice and 2 years in rats (WHO, 1990) [30]. Typically the rat weanlings or post-weanlings have been used for a combined chronic toxicity/carcinogenicity assessment [31]. Ideally, the design and conduct of the test should allow for the detection of neoplastic effects and a determination of carcinogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related

Table 6: parameters to be evaluated as a part of OECD test guidelines.

Characteristic	*Parameter to evaluate*
Hematology	• Haematocrit,Haemoglobin concentration • Erythrocyte count, Total and differential leukocyte counts ,Platelet count, • Erythrocyte morphology, • Mean corpuscular volume, mean corpuscular hemoglobin, Mean corpuscular hemoglobin concentration, • A measure of blood-clotting potential.
Chemical pathology/clinical biochemistry (Major toxic effects in tissues such as the liver, kidney and heart are assessed)	• Sodium , Potassium, Glucose, • Total cholesterol, Urea, Creatinine, • Total proteins and albumin, • At least two enzymes indicative of hepatocellular effects SGPT/SGOT, γ-glutamyl transpeptidase, lactate dehydrogenase and sorbitol dehydrogenase), • Urine analysis on appearance, volume, osmolality, Specific gravity, pH, protein, glucose and blood cells.
Clinical and functional observations (once a day observation is necessary but recordings for morbidity and mortality should be done at least twice daily)	• Changes in skin, fur, eyes, mucous membranes, occurrence of secretions and excretions and autonomic • Changes in gait, posture and response to handling as well as the presence of clonic or tonic movements, • Stereotypes (e.g. excessive grooming, repetitive circling) or bizarre behavior (e.g. self mutilation), • sensory reactivity to stimuli of different types (e.g. auditory, visual and proprioceptive stimuli, assessment of grip strength (Meyer et al., 1979) and motor activity assessment (Crofton et al., 1991)
Toxicokinetics and metabolism	• Rate and pattern of absorption and distribution in tissues, organs and fluid compartments, • Reversible binding to tissue sites and plasma proteins, • Pattern and rates of metabolism and excretion profiles.
Teratology	• Mating behavior, • Percentage of females pregnant, • Number of pregnancies going to full term, • litter size, • Number of live births, • Number of stillborns, • Pup viability and weight at parturition, and postnatal days 4, 7, 14 and 21 days of age, • Fertility index, • Gestation index, • Viability index, • Lactation index • Sex ratio of fetuses, • Fatal/litter weights, • Number and percentage of fetuses/litter with malformations and variations.
Geno/neuro toxicity	• Bacterial reversal mutations tests (e.g. Ames test), • Tests in mammalian systems (e.g. chromosomal aberration tests, • erythrocyte micronucleus test • sister chromatid exchange assay unscheduled DNA synthesis), • In-vitro gene mutation assays in yeast, Dominant lethal test in rodents and the mouse spot test. • Comet assay (DNA damage and repair), • Mutations in transgenic animals, fluorescent in-situ hybridization and cell transformations. • Functional tests (e.g. auditory, visual), Ophthalmological examinations, Limb grip strength [1], Motor activity[2], Incidence of specific neurobehavioral neuropathological, neurochemical or electrophysiological abnormalities.

1. Meyer OA, Tilson HA, Byrd WC, Riley MT (1979). A method for the routine assessment of fore and hindlimb grip strength of rats and mice. Neurobehav Toxicol 1: 233–236.
2. Crofton KM, Howard JL, Moser VC et al. (1991). Interlaboratory comparison of motor activity experiments: implication for neurotoxicological assessments. Neurotoxicol Teratol 13: 599–609.

morphological effects. Preliminary studies providing data on acute, sub-chronic and toxicokinetic responses should have been carried out to permit an appropriate choice of animal species and strain (selected strains should not have a high spontaneous background tumor incidence). Dosing of the rodents should begin possibly after weaning and acclimatization, and preferably before the animals are 6 weeks old. Refer to table-5

Developmental and reproductive toxicity studies

The guidelines and procedures for developmental and reproductive toxicity studies are well documented in Korach[32] (1998) as well as the OECD guidelines 414–416. These studies could be one generation (OECD 415)[33], two-generation (OECD 416)[34] or three generation tests. Developmental toxicity studies (also called teratology studies) are designed to look at a wide spectrum of possible in utero outcomes for the conceptus, including death, malformations, functional deficits and developmental delays in fetuses. Refer to table-5

Genotoxicity and neurotoxicity studies

These are designed to determine whether test chemicals can perturb genetic material to cause gene or chromosomal mutations. A large number of assay systems, especially in-vitro systems, have been devised to detect the genotoxic or mutagenic potential of agents. Refer to table-5

Conclusion:

Establishing the toxicological profile of ayurveda, siddha drugs and formulations can be accomplished satisfactorily by employing the aforementioned established toxicological protocols .Testing via established protocols accounts for its data veracity and reliability .since very little toxicological data is available and the data for the contentious herbo-metallic compounds are yet to be determined in definitive terms , employment of concurred-upon OECD and comparable protocols would facilitate a wide spread dissemination and use of herbal products which are of late plagued by want of credible and reproducible data. These data can only be obtained by employment of protocols prescribed by cGLP [35] norms of prominent regulatory agencies. Thus getting actionable toxicological data for ayurveda and siddha drugs and formulations would be a fillip to wide spread acceptance.

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