HERBAL VETERINARY DRUGS: PHARMACOLGICAL VALIDATION AND VALUE ADDITION

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Herbal veterinary drugs are used worldwide particularly in developing countries to treat and reduce sufferings of humans and animals and to improve their productivity since antiquity. Sixty five per cent of world population depends almost entirely on plants for medication (Fabricant and Farnsworth, 2001). No specific estimates of medicinal plants in animal healthcare are available, however, it is expected to be still very high. India had a rich heritage of using medicinal plants in Ayurveda, Unani, besides folk use. First mention of plant drugs is found in Rigveda and Yajurveda (2000 BC). The extent of herbal drug usage is difficult to quantify for indigenous system of medicine in India, as by tradition, village folk prepare their own prescription for animal treatment, however, there are around 37 Pharmaceutical houses producing one or more herbal drugs in India. Major veterinary herbal drug-producing companies are around 10. In India, the total animal healthcare product market is estimated at rupees 1500 crores. The Herbal animal healthcare product share is believed to be only 200 to 225 crores (13-15% of total market) (Agarwal, 2004).

Herbal drugs were the most important drugs in the treatment of diseases until 19th century, leading to isolation of active constituents with precise pharmacological activity. During and after World War II, with the development of synthetic drugs, medicinal plants with great therapeutic potential were forgotten. In the modern days, animal healthcare relies heavily on allopathic system of medicines. However, indigenous system survived due to availability of major population of animals in villages and villagers use plants for their folk use. This ensured continued presence and usage of herbal products in veterinary care in India. Reversal to herbal drug is due to the fact that in synthetic drugs, there are problems, which include high cost, non-availability in rural areas, toxicity, environmental pollution, resistance development and chemical residues. Over and above, reliance on synthetic drugs does not invalidate herbal drugs. With regard to toxicity of synthetic drugs, diclofenac sodium toxicity in vultures is a glaring example of toxic effects of synthetic drugs. Diclofenac sodium used as anti-inflammatory and analgesic in livestock has been implicated in the toxicity of vultures (Oak *et al.,* 2004), resulting in its declining population to extinction.

Taking cognizance of these reasons, there had been a great scientific interest in evaluation of indigenous medicinal plants in the treatment of various diseases. The search for safe and efficacious herbal drug may overcome some of the problems associated with synthetic drugs. Furthermore, there is feeling that herbal drugs are safe because they are more in harmony with the biological system. Concerted research efforts are needed in this direction in our country. In Western world too, there is upsurge of interest and reliance in herbal drugs/formulations. In Germany, the safety of long-used natural products is generally assumed if no side effects have been reported and heavy emphasis is placed on the reports of general practitioners and extensive clinical trial are not required (Abelson, 1990).

More than 100 drugs of contemporary medicine are extracted from higher plants and as such plant drugs deserve detailed studies in the light of modern science. The plethora of chemical and biological tests, including high-throughput screening makes development of herbal-based novel drugs an area and appropriate time for research. Since plants have provided many drugs in the past, and they remain a rich source of novel compounds based on Nature's combinatorial natural products chemistry over millions of years of evolution, they should continue to be investigated as a source of novel therapeutic agents. The majority of the plant species has not been investigated chemically or biologically, and bioassay-guided fractionation, dereplication techniques and powerful methods of structure determination will continue to help this research in future. It may be predicted that there will be continued demand of high quality, safe and effective herbal medicinal products (HMPs) also requiring continued scientific investigation (Cragg *et al.,* 1997; Phillipson, 2003).

A large number of plant/plant material of doubtful utility are being used globally in traditional system of medicine. The world is continuously losing plant species through agriculture and urbanization and the knowledge based on the use of indigenous medicinal plants is constantly being eroded. Therefore, the scientists need to catalogue the actual use of medicinal plant species.

India has 15 Agroclimatic zones, 47,000 different plant species and 15,000 medicinal plants. The Indian Systems of Medicine have identified 1500 medicinal plants, of which 500 species are mostly used in the preparation of drugs. The medicinal plants contribute to cater 80% of the raw materials used in the preparation of drugs. The effectiveness of these drugs mainly depends upon the proper use and sustained availability of genuine raw materials, followed by evaluation using modern scientific methods and tools, *viz.,* chemical, biochemical, biotechnological, pharmacological, toxicological and pathological for rational drug development.

India has a great potential in the trade of herbal-based drugs. Thus, it is the need of time that well validated and value added products are used in the indigenous and foreign market. Validation is must as there is lack of data on reproducibility of effects, lack of data on controlled clinical trials, lack of recognition of active fraction/active principles and lack of data on interaction with food and synthetic drugs and toxicity.

Validation of herbal drugs should follow the following serial steps:

1. Information about the herbal remedies could be gathered from traditional uses, practiceners, healers, farmers, nomads, authentic literature, etc.
2. Such herbal remedies should be discussed in detail with panel of experts for primary screening, involving herbalists, field veterinarians, pharmacologists, pharmacognosists, etc for their valuable use for the purpose they are being reported by the users and only those remedies which seem worthy could be further processed for validation.
3. Identification of plants/ plant material: Plants/ plant parts with therapeutic potential in various ailments should be collected from suitable source and botanically identified.
4. Properly identified plant/ plant material should be processed as per its traditional guidelines for preparation of remedy and then used in those proven clinical cases and similar experimental conditions for which the remedy has been prescribed.

5. Now those remedies that have shown their efficacy in clinical and or experimental conditions should be finally selected and their ingredients again confirmed.
6. Extraction of active fractions: Identified plant material should be air-dried, grounded and defatted with petroleum ether. The defatted material should be extracted with suitable solvents to obtain different fractions.
7. Different fractions of various plants should be subjected to respective biological activity and evaluation to identify the fraction, possessing promising activity *in vitro/in vivo* systems.
8. Purification of active fractions: The active fraction thus obtained/identified should be subjected to chemical group analysis with the help of suitable techniques.
9. Chemical characterization of active principles should be undertaken at an advance center earmarked for the purpose.
10. Pharmacodynamic studies of the active fractions/principles (*in vitro* and *in vivo*) should be carried out.
11. Toxicological evaluation of active fractions/principles should also be undertaken in active collaboration with Veterinary Toxicologist and Pathologist.
12. Clinical evaluation of active fractions/principles should be carried out with the help of clinicians in multi-centre clinical trials.
13. Fractions/active principles, found to possess the promising activity could be subjected for development of drug/formulation in consultation with pharmaceutical establishment.

Rational pharmacological evaluation for the pertinent activity is the very important aspect apart from various steps involved in the validation of herbal veterinary drugs. There are more than 50 distinguished therapeutic categories for which herbal drugs (drugs) can be employed. Following table provides list of some procedures for evaluation of pharmacological properties of herbal materials.

A. Methods and animals used for evaluation of various pharmacological activities *in vivo*:

SNo.	Activity	Method	Experimental Animal
1	Analgesic	Randell-Salitto Assay	Rat
		Writhing test	Mouse
		Tail clip test	Mouse
		Hot plate test	Mouse/rat
		Tail-flick response	Mouse/rat
		Tail immersion test	Mouse/rat
2	Anti-allergic	Passive cutaneous anaphylaxis test	Mouse/rat
		Schultz-Dale phenomenon	Guinea pig
		Eye model of allergy	Guinea pig
		Compound 48/80-induced mortality test	Rat
		Skin permeability (autacoid test)	Rat

		Mast cell study: a) degranulation of mast cells b) compound 48/80-induced histamine release	Rat
		Bronchodilator test: a) Bronchial muscle *in vivo* b) Allergic asthma and histamine aerosol test	Guinea pig
3	Anti-arrhythmic	Electrical/chemical/mechanical-induced arrhythmia	Cat
4	Antibacterial	Disc diffusion technique	*In vitro*
		Growth/tube dilution technique	*In vitro*
		Micro titre technique	*In vitro*
5	Anti-fungal	Corneal infection	Rabbit
		Vulvo-vaginal candidiasis	Mouse
		Systemic candidiasis/aspergillosis	Mouse
		Pulmonary cryptococcosis	Mouse
		Broncho-pulmonary aspergillosis	Monkey
6	Anti-hypertensive	Neurogenic hypertension	Rat/rabbit/dog
		Dietary hypertension	Rat/chicken
		Renal hypertension	Rat/rabbit/dog
		Genetic hypertension	Rat (Kyoto strain: SH rat)
7	Antiinflammatory	Carrageenan-induced oedema	Mouse, Rat
		Cotton pellet test	Rat
		Granuloma pouch test	Rat
		Formaldehyde-induced arthritis	Rat
		Adjuvant-induced arthritis	Rat
		Collagen-induced arthritis	Rat
		Stabilization of lysosomes Experimental pleurisy	Rat
8	Antipyretic	Yeast-induced pyrexia	Rat
9	Anti-secretary	Continuous recording of gastric secretion	Rat
10	Anti-thrombotic	Extracorporeal shunt model	Rat
		ADP-induced electrocardiogram alteration	Rat
		Collagen and adrenaline induced pulmonary thrombo-embolism	Mouse

		Venous thrombosis	Rat
		Stasis induced venous thrombosis	Rat
11	Anti-ulcer	Pyloric ligation	Rat
		Restraint ulcer	Rat
		Drug-induced gastric mucosal damage	Rat
		Histamine-induced gastric ulceration	Guinea pig
12	Central Nervous System	Spontaneous motor activity	Mouse
		Forced locomotor activity	Mouse
		Amphetamine induced hyperactivity	Mouse
		Hexabarbital sleeping time	Mouse
		Conditioned avoidance response	Rat
		Amphetamine toxicity	Mouse
		Anti-reserpine test	Mouse
		Swimming performance test	Rat
		Anti-convulsant activity	Mouse
		Pentylenetetrazole seizure threshold test	Mouse
13	Hypoglycaemic	Alloxan-induced diabetes	Rat/monkey/rabbit
		Pancreatectomy-induced diabetes	Rat
		Streptozotocin-induced diabetes	Rat
		Glucose loading	Rats
14	Hypolipidaemic	Triton-induced hyperlipidaemia	Rat (Charles) Forster strain, male adult
		Diet-induced hyper lipoproteinaemia	Rat (Charles) Forster strain, male adult
		Cholesterol biosynthesis	In vitro
		Antilipolytic activity	In vitro
		Inhibition of platelet aggregation	In vitro
15	Hepatoprotective	Carbon tetrachloride-induced hepatotoxicity	Rat
		D-galactosamine-induced hepatotoxicity	Rat
		Plasmodium berghei infection	
		Paracetamol-induced hepatotoxicity	Rat
		Thioacetamide-induced hepatoxicity	
		Monocrotaline-induced hepatoxicity	
		Aflatoxin B1-induced hepatoxicity	

B. Experimental pharmacological techniques involving anaesthetized animals:

These techniques require anaesthetized animals for evaluation of some important physiological activities.

i. Studies on spleen volume, blood pressure and respiration of dog

ii. Studies on blood pressure and nictitating membrane of cat

iii. Studies on decerebrated cats

iv. Studies on superior cervical ganglion and nictitating membrane of cat

v. Studies on hindquarters perfusion in the intact anaesthetized cat

vi. Studies on digoxin-induced arrhythmias in dog

C. Isolated tissue preparations

Isolated tissue preparations have been in use for last many decades and many important findings have been made out of the experiments conducted on them. One of the major advantages of Isolated tissue preparations is that they can be easily set up and used for evaluating a large number of test materials without being influenced by various pharmacokinetic factors. Some routinely used isolated tissue preparations are listed below.

SNo.	**Isolated tissue preparation**	**SNo.**	**Isolated tissue preparation**
1.	Cat splenic strip	22.	Isolated perfused intestine
2.	Cat right ventricular papillary muscle	23.	Isolated perfused pancreas
3.	Cat tibialis anterior muscle nerve preparation	24.	Mouse ileum
4.	Cat gastrocnemius-sciatic muscle nerve preparation	25.	Mouse vas deferens
5.	Frog *rectus abdominis* muscle	26.	Rat ileum
6.	Guinea pig myentric plexus-longitudinal muscle	27.	Rat stomach (fundus)
7.	Guinea pig hypogastric nerve-vas deferens preparation	28.	Rat duodenum
8.	Guinea pig tracheal chain	29.	Rat uterus
9	Guinea pig ileum	30.	Rat anococcygeous muscle
10.	Guinea pig ileum	31.	Rat phrenic nerve diaphragm
11.	Guinea pig seminal vesicle	32.	Rat ascending colon
12.	Guinea pig taenia coli	33.	Rat descending colon
13.	Guinea pig atria	34.	Rat vas deferens
14.	Guinea pig right ventricular papillary muscle	35.	Rabbit ileum/jejunum
15.	Hamster stomach strip	36.	Rabbit duodenum
16.	Isolated perfused heart	37.	Rabbit aortic strip

17.	Isolated perfused liver	38.	Rabbit perfused ear artery
18.	Isolated perfused lung	39.	Rabbit atria
19.	Isolated perfused kidney	40.	Isolated goat uterus
20.	Isolated perfused brain	41.	Isolated sheep pulmonary artery
21.	Isolated fowl carotid artery	42.	Isolated rat portal/mesenteric vein

D. Cell Culture Preparations

Cell culture preparations are also used frequently in the area of pharmacology and toxicology. Various cell culture systems including freshly isolated cells (hepatocytes, intestinal cells, reticulo-endothelial cells, etc.) in suspension and primary cultures and cells in permanent culture are now available with a variety of methods for evaluation of drugs. Apart from easily manageable cultures of less differentiated cells, organ-specific cultures of most specialized cells such as hepatocytes, nerve cells and skin cells, etc. are also employed to evaluate the effect of drugs on corresponding targets in the body. Following are some of the established mammalian cell lines being used for pharmacological studies.

H-4-II-E (hepatoma rat)	SV 40 (lung, human)
BHK21 (C13) (kidney, golden hamster)	AD-108 (lung, Chinese hamster)
GHK-TK (kidney, golden hamster)	CC164 (lung, mink)
RAG (Renal adenoma, BALB/C mouse)	L-A9 (fibroblast, mouse)
LLC-PK (kidney, pig)	NC37 BqaEV (lymphoblastoid, human)
Vero (kidney, African green monkey)	HepG2 (Human hepatoblastoma –F111 cell line-basal and inducible levels of mono-oxygenase)
CV1P (kidney, African green monkey) (fibroblast, cat)	MH_1C_1 (rat hepatoma cell line-C81 basal inducible levels of mono-oxygenase)
3T3/BALB (fibroblast, mouse)	7777 (rat hepatoma cell line-Hela cell line) basal and inducible levels of mono-oxygenase
A549 (pulmonary carcinoma) (lymphoma, mouse)	HTC (rat hepatoma cell line-no-S49 mono-oxygenase activity)
G-361 (melanoma, human) (neuroblastoma, mouse)	JM2 (rat hepatoma cell line-C-1300 basal and NO mono-oxygenase activity)
C-6 (glioma, rat) (macrophages, mouse)	Hepa IcIc 7 (murine hepatoma –IC-21 cell line-basal and inducible levels of mono-oxygenase)
CHO (ovary, Chinese hamster)	3T3 L1 cell line
V79 (lung, Chinese hamster)	SIRC (Rabbit corneal cell line)
MRC-5 (lung, human)	A431 human epidermal cell line
WI-38 (lung, human)	

Value addition:

Herbal drugs can be produced from medicinal plants in several forms, depending upon the requirement in the traditional and modern systems of medicine and the degree of sophistication of the preparation methodology. Value addition can be carried out based on knowledge for the purpose of exporting value-added material rather than raw material. They could be crude plant material, standardized plant extracts, partially purified extracts, group of phyto-chemicals to the pure single phytochemicals. The extent of value addition carried out at the beginning could be cleaning, drying, and sorting of natural products, which are normally required by local Dealers. At Dealers' level, value addition could be limited by industrial buyers' quality standards and government regulatory authorities for which, often identification and chemical analysis could be conducted in laboratories in order to meet the standards laid by regulatory authorities through industrial participation with government providing the nucleation.

Some degree of technological intervention is needed for value addition, depending on the type of extract required for production of herbal preparation at larger scale. Various dosage forms can also be formulated that may improve utility. To herbal products value addition can further be done by purification of extracts and isolation of single bioactive molecules. As India has a large number of tried and time-tested herbal products in use, vigorous quality control, proper packaging and a brand name is required to withstand international market competition.

Conclusion:

Validation of herbal drugs is beneficial in terms of developing economical, eco-friendly, easily accessible, effective and safe drugs for enhancing livestock health and production. Negative validation will save the livestock owners from practising ineffective remedies. In addition to development of new drugs with divergent therapeutic potential, the process may lead to unravel a good pharmacological tool quite often. However, it is felt while validating herbal veterinary drugs that suitable test systems are not available. Nevertheless, validation and value addition will not only contribute to support health and production for our vast livestock, but it will also boost the trade of herbal drugs.

Further readings

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