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SAFETY AND EFFICACY OF HERBAL REMEDIES: A REVIEW OF THE MODELS FOR VALIDATION OF HERBAL REMEDIES OF SOME NEUROPHARMACOLOGICAL CONDITIONS.

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Abstract

Background: Herbal remedies are making waves in many neurological conditions, and it will be wrong to assume that they do not have to be subjected to the same rigorous ethical investigational pathways as for the synthetic medicines/remedies. The primary and most important concern of pharmacologists in the team of drug developers is the safety of the remedy, whether herbal or synthetic. The remedies are aimed at the human body for the alleviation of the medical condition, so it makes sense to protect that body against further injury. In this context, there is no consideration for a different treatment when herbs are involved.
Methods: This review is based on the teaching approaches of the author, with a view to explaining the rationale for some of the experimental steps in neuropharmacological experiments, particularly with herbs. The issues of experimental models are discussed, with sufficient explanations for the choice of the model, the indices to monitor and the interpretation of the indices. Supporting literature are also provided as appropriate.
Conclusions: Appropriate conclusions are drawn and the target audience are put in a good stead of the appreciation of why they do what they do, while correcting what they have not done well.

Keywords: validation; models; herbal medicines; neuropharmacology; disorders

Introduction

Herbal resources are fast gaining grounds in addressing medical disorders, including neuropharmacological disorders (Ekor, 2014). This is particularly true in refractory epileptic conditions, where a significant number of the antiepileptic synthetic drugs are either ineffective or laced with side effects that discourage adherence; in addition to cost that may be prohibitive (Raucci *et al.*, 2020; Osuntokun *et al.*, 2022). Other neuropharmacological disorders where herbs have been and are being investigated for improved outcomes include depression, insomnia, inflammation, anxiety, learning and memory etc (Akanmu *et al*., 2005; Akinpelu *et al*., 2017; Akinpelu *et al*., 2019; Adegbuyi *et al*., 2020). At the early stages of documentation of herbal resources for treating/managing medical conditions, the World Health Organization (WHO) gave what appears like a waiver to encourage the development of herbal remedies (Ekor, 2014). The waiver came in a statement by WHO that "herbs that have been used in a culture for a long time, with proven safety and efficacy can be safely adopted for continued use" (Welz *et al.*, 2018). This fairway by WHO is the focus of this review. Time has changed and the people have also shifted from what made WHO to give that waiver. The caveat that WHO embedded in the waiver is currently abused, especially in Africa, Nigeria in particular. The world has developed technology that can ensure and assure safety and potency of herbal remedies (Grollman & Marcus, 2016); while the honesty indices have also shifted south, due to unemployment, illiteracy, uncontrolled population explosion, drug enforcement failure and corruption. With advancement in knowledge of the theories behind how the herbs work, specifically on the possible mechanisms of action, elucidated through scientific probes and validations, there is no excuse for exception and waiver (Zhang *et al.*, 2011). The waiver which was also due to the level of scientific knowledge at the time, and the uncertainty of the application of the same process and procedure that was used for the validation of synthetic drugs, is finally being harmonized (Vinarov *et al.*, 2021). It is therefore time to interrogate the

WHO waiver and insist that the apparent dichotomy between the synthetic drugs and herbal remedies be unified. This will ensure safety, increase cost effectiveness and promote across board, acceptance of herbal resources for the treatment or management of ailments. It is instructive to note that the excuse for the grant of leeway for herbal remedies not to be subjected to rigorous scientific probe in the past was partly because of the consideration for the poverty level of Africa and other primordial cultures. However, today, herbs do not come cheap and may not be cost effective on the long run; and the poverty index for the poor countries is still looking north (Alostad *et al.,* 2018). Spurious claims about efficacy, including unscientific, unverified and unverifiable claims such as cure for hypertension and diabetes are prominent. This review attempts to bring to the fore, the ramifications of experimental models as applicable to neuropharmacologically active herbs and by extension, to all medical conditions. The discourse will cover aspects of animal choice, animal care and handling, choice of models, treatment of herbs for experimental purposes, choice of doses and guiding principles, indices to score, interpretation of quantitative scores, determination of mechanism(s) of action, statistical inferences, layout of report and summary.

Animal choice

The very first good step in neuropharmacology is the identification of the animal to experiment with. In nearly all neuropharmacological experiments, except inflammation investigation, where rats are deployed, mice are the animals of choice (Ahmed, 2017). The choice of mice is pragmatic as mice are active ordinarily. The indices to measure are better with mice (rearing, grooming, face wash, stretch attend, walling, defecation, urination) in terms of frequency of occurrence (Hill *et al.,* 2018). Rats may not engage in rearing spontaneously, as they may not jump spontaneously in the hot plate experiment for anti-inflammatory/analgesic evaluation (Deuis *et al.,* 2017). These behavioural indices are the necessary first step in neuropharmacological experiments because they give direction to further investigations (d'Isa *et al.,* 2021). A reduced rearing index is interpreted to mean a central nervous system (CNS) depression while increased rearing indicates CNS stimulation. It should be noted that big rodents are not employed at this stage of experimentation because the preliminary purpose are not served by the big rodents. Choice of animal is largely influenced by the type of study. For sedation, convulsion, psychosis, learning and memory etc, mice are deployed. For studies involving paw pressure measurements as we have in inflammatory studies, it is more rewarding to use rats because of convenience of size. It is also instructive at the initial stages of the investigation to utilise male animals because of issues of pregnancy and hormonal interference with studies (Turner *et al.,* 2011). Adult animals are also used, except where developmental issues are being investigated. Adulthood in animals is correlated with weight (18-30g for mice and 150-250g for rats) (Sengupta, 2013). Most researchers into herbal remedies do not breed their animals in their facilities+, so the records of birth, and therefore age may not be available. It therefore suffices to use weight.

Table 1: Appropriate animal for each neuropharmacological model

Model type	**Animal**
Sedation/Stimulation	Mice
Convulsion	Mice
Inflammation (paw size)	Mice or rats
Inflammation (writhes frequency)	Mice
Inflammation (analgesiometer)	Rats or mice
Learning and memory	Mice
Anxiety	Mice
Antipsychotic	Mice
Antidepressant	Mice

Animal care and handling

The care of the experimental animals is critical. Animals for neuropharmacological experiments must be healthy, because one cannot deploy a sick animal at any stage of animal experimentation (Hajar, 2011). Kindly note this as different from the phases 2 and 3 of human clinical trial, where persons having the target medical condition may be deployed for the trial (Van-Norman, 2019). Use of sick animals during investigation may apply in veterinary practice. Indeed, every reputable publishing house/journal insists on ethical clearance for the use of laboratory animals (Simmonds, 2018). Animals for neuropharmacological investigations must be acclimated to the laboratory environment prior to the commencement of the investigation. Handling is very germane in neuropharmacology. Agitation should be avoided in animals to be studied to avoid false positive or false negative results. Agitation will elicit response from mice and rats that may lead to bites, urination and defecation. Small rodents may be placed on sleeves or pockets of laboratory coats while getting them ready for application of extracts. Whether the test herbal extracts are administered orally or intraperitoneally, the animals are held by the scruff. This makes it impossible for the animal to turn or twist to bite the handler. For intraperitoneal administration of extracts, the animals are held with the head pointing south (down),

this is to ensure that the visceral organs are free from injury from the needle. It is interesting to note that some inexperienced animal researchers may puncture the visceral organs and the animal may go into distress by exhibiting signs misinterpreted as sedation or toxicity (Dogrul *et al.,* 2020). Home cages should be cleaned daily, with replacement of the wood shavings. Animals in observation cages, especially when urine drops as well as fecal drops may be of interest to the researcher, may not have wood shavings. In neuropharmacological experiments, particularly behavioural studies, animals are used only once (Sjoberg, 2017). The observation cage (plexiglass material, also called open field or arena) is cleaned after each observation to annul lingering olfactory cues that may influence the behaviour of incoming experimental animal. This is done by using 70% alcohol (Lehmkuhl *et al.,* 2014).

Choice of models

Models are approximate representation of the medical condition of interest to investigate. The choice of model needs some experience and here is where mentorship shows. More than one model can be used to deliver a particular investigation, with varying levels of sensitivity (Straus *et al.,* 2013). A model can be tangible, as we have with rotarod; can be intangible, using only drugs as we have with convulsion models; or a combination of both as we have with anxiolysis, learning and memory investigations (Marshall *et al.,* 2021). Some of the models are described in detail.

1. Sedative model

In neuropharmacological investigations, it is common to use a plexiglass box of fixed dimension (25cm X 25cm X 30cm for mice) (Kestering-Ferreira *et al.,* 2021) called the open field or arena. The arena is a regular box made of plexiglass for ease of cleaning, and made of opaque colour on all sides except one side for visual observation and recording of activities like rearing, grooming, stretch attend, walling, line crossing, wet dog shake, genital licking, defecation, urination (Olayiwola *et al*, 2007). The observation of the activities of the animal should be discrete so that the animal is unaware of the observation (Larsen *et al.,* 2017). Ambient condition is created for the experiment; indeed, the laboratory temperature, humidity and light intensity should be standardized and documented for reproducibility (Russell *et al.,* 2021). Animal independency for behavioural observation should also be advocated by the use of camera or the observer should be far from the animal during observation. The farther the observer is from the animal the better the behavioural outcome.

2. Anticonvulsant model

This model naturally follows the sedative model. It should be noted that depending on the score in the sedative model, the anticonvulsant possibility of the herbal extract can be explored. If the extract is pointing in the direction of a sedative; having been recording a decrease in rearing, grooming, face wash and other CNS depression indices, it should be investigated for possible anticonvulsant effect (Olayiwola *et al.,* 2013). The anticonvulsant model utilises chemicals mostly. The same arena is used as for the sedative experiment above. The anticonvulsant effect can be tested using two (2) models: the chemoconvulsion and electroconvulsion models (Holmes & Zhao, 2008). The chemoconvulsion is done through three axis: the pentenyltetrazole (leptazol), picrotozin, bicuculine, yeast axis that utilises the Gabaergic receptor axis; the glutaminergic pathway that utilises NMDA and the strychnine that utilises the glycine receptor axis (Estrada-Mondragon and Lynch, 2015). The import of this is that as the extract is investigated for possible blockade of the above convulsants, the receptor by which the extract is working is simultaneously unraveled (Citraro *et al.,* 2016). The electroconvulsion is a general model to investigate the anticonvulsant effect of an extract without the additional benefit of receptor elucidation that the chemoconvulsants have (Duthie *et al.,* 2015).

3. Learning and memory

The battery of tests for learning and memory are many and varied. Prominent among them is the Morris water maze (Vorhees & Williams, 2006). Others are the Y-maze, T-maze, Barnes maze, Oasis maze and radial arm maze models (Kraeuter *et al.,* 2019). Novel object recognition test may also be employed to study learning and memory (Antunes & Biala, 2012). Other models include, but not limited to: Cincinnati water maze, delayed match to place water maze, fear conditioning, intellicage place learning and cue discrimination experiments, object location memory task, passive avoidance task, satellite box exploration in the intellicage (Stanford medicine, 2022.). Here, memory inhibiting drugs and substances may be employed to see the reversal of the inhibition by the test herbal remedy. Examples of the chemically induced memory impairment models include scopolamine, MPTP (1-methyl-4-phenyl-1,2,3,6-Tetrahydropyridine), 6-Hydroxy Dopamine, Amyloid Beta-Peptide, ethanol, colchicine, Streptozotocin, Quinolinic Acid, 192 IgG-Saporin, Okadaic Acid, Domoic Acid, Trimethyltin, Ethylcholine Aziridinium, Ibotenic acid and some metals *(*copper (Cu), chromium, cobalt, aluminum (Al), iron, zinc (Zn) lead (Pb), arsenic (As) and cadmium*)* (More *et al.,* 2016). In these plethora of models for learning and memory, the choice of the one to use is influenced by experience, convenience and the objective of the study.

4. Antidepressant model

Antidepressant investigation models include the forced swim test, the despair study in mice (suspended mouse by the tail). The model is hinged on the extent of endurance of rodents to hold on to life, to escape or vocalize despair, and the experiments usually take place in environments that are not normal habitats of the rodents (Bryda, 2013). Indeed, environments that they avoid, like water. The plant extract is expected to extend the endurance time, when compared with untreated animals (negative control); and indeed be comparable to those treated with the standard antidepressant drugs (Guerrera *et al.*, 2020). Other models such as reserpine-induced depression, yohimbine-induced lethality test, chronic unpredictable mild test and others.

5. Anxiolytic model

Models used to establish the effectiveness of potential anxiolytic plants include the open field, elevated plus maze, the head dip apparatus, Y-maze and Barnes maze (Harquin *et al.*, 2014). Anxiety is a complex phenomenon in humans, presenting many facets like generalized anxiety, social anxiety, panic, post-traumatic stress disorder, and phobia. The first challenge is characterizing the type of anxiety and correlating it with the animal model deployed (Bandelow & Michaelis, 2015). Also, different stimuli modulate the genesis of anxiety in animals. Few examples include, but not limited to whether the anxiety is generated by acute or chronic stress, spontaneous or conditioned responses (Lovick and Zangrossi, 2021). The same applies to receptor/agonist/antagonist deployment in anxiolytic elucidation and validation. While some respond to benzodiazepines, some respond to serotoninergics, affirming the complexity of anxiety as a phenomenon (Guina & Merrill, 2018). Even within the serotoninergic receptors, 5-HT subtypes account for difference in response of animals, with some supporting anxiolysis and some not (Guina & Merrill, 2018). Meta-chlorophenylpiperazine (mCPP), a 5-HT_{2c} partial agonist, was documented to be anxiogenic while ritanserine and ketanserine (nonselective 5-$HT_{2a/2c}$) are anxiolytic (Thomas *et al.*, 2018). For any model, but particularly for animal model of anxiety, three characteristics establish validity of the model. It is then imperative to choose a model with most of the indices of the validity. One is face validity, second is predictive validity and thirdly, the criterion of construct validity (Belzung & Lemoine, 201). The face validity holds for the animal model to a great extent except for the lack of vocalization in animals, a feature that is present in normal/regular humans. The predictive validity has to do with the extent that the data is predictable of the clinical setting. In this way, the mice and rats that are the rodents of choice because of availability and cheapness, only approximate to clinical setting. Here pharmacological agents should produce similar effects in the models and humans. The construct criterion has to do with the correlation of the theoretical rationale underlying the animal model and the human behaviour being investigated (Planchez *et al.*, 2019).

6. Antipsychotic model

The construct validity hold true for the antipsychotic model as the most deployed model is the stereotyped behaviour model and the paw test model. The stereotyped model utilizes either amphetamine, methlyscopolamine or apomorphine to cause a repetitive movement of the head region, including gnawing in an involuntary manner. This is akin to being psychotic in rodents (Olayiwola *et al.*, 2013; Forrest *et al.*, 2019; Osuntokun *et al* 2022). The paw test is premised on the forced push of the forelimbs and the retraction of the hind limbs; movements that approximate in rodents to psychosis in humans (Mazarati *et al.*, 2018).

7. Anti-inflammatory model

Models for the investigation of the potentials of some extracts to remedy inflammation are premised on the creation of inflammation in the experimental animals and the evaluation of the extracts for prevention of the sequelae of inflammation syndromes (Patil *et al.*, 2019). They are simple models, sometimes requiring no sophisticated equipment, indeed requiring no equipment at all. For convenience, the evaluation of the analgesic potential of an extract will be taken here, partly because the practical basis of analgesia is somewhat similar or close to what obtains with inflammation (Guo *et al.*, 2018). While pain is the denominator in both conditions, the analgesia and inflammation investigative protocols are evaluated via pain indices. In practical terms, pain and inflammation can be instituted in three ways; thermal, chemical and mechanical (Reker *et al.*, 2020). The quantitative and quantifiable indices of inflammation include the increase in paw size as a result of oedema induced by carrageenin, yeast, acetic acid etc (Mansouri *et al.*, 2015). These form the chemical approach. Inflammation can also be assessed thermally, using the hot plate method and assessing the endurance or time to escape (Deuis *et al.*, 2017). For the mechanical pain assessment, an analgesiometer is used. This apparatus delivers gradual pressure on the hind paw of the animal until the animal vocalizes pain or withdraws the limb (Houston *et al.*, 2021). Appropriate pressure is read from the apparatus, in the absence of treatment and in the presence of treatment with both test extract and standard analgesic/anti-inflammatory agent.

In the thermal experiments, it is imperative that the temperature of experiment is regulated, because there is difference in the interpretation of the reaction to 51℃ and less than 51℃ (Cheung, 2015). It is also increasingly being appreciated and recommended that the hot plate model utilizes mice rather than rats because mice have a linear reaction

to the thermal threshold while the rats have a somewhat complex reaction. While mice will jump when the thermal threshold is attained, rats may choose to lick the paws or engage in grooming instead of jumping (Navarro *et al.,* 2021).

The chemically induced writhes with the aid of acetic acid makes the mouse stretch significantly, and repeatedly. Recall that stretching is a common behaviour of rodents. Indeed, experiments with rodents utilize the extension or abolition of their natural behaviours (Bayne, 2018).

Handling of herbs for experimental purposes

There has been a lot of conversations about how herbs are handled for experimental purposes (Ekor, 2014). A school of thought believes that herbs should be investigated in the format that the community uses them. Very reasonable, except that such format may not be scientifically expedient. The scientist is interested in the active principles as well as the mechanism(s) of the demonstrated activities. These can only be achieved if we follow the scientific course-way, including the protocols, which together ensure reproducibility. Reproducibility is the hallmark of science. Therefore, the conversationalists for using herbs as it is used in the population will not have validation on their side. The way to go is to subject the herbs to treatments that ensure the hallmarks of science (Welz *et al.,* 2018).

Herbs when freshly harvested are air dried and not sun dried, to prevent the destructive effects of the UV rays from the sun (Shonte *et al.,* 2020). There is no rule as to the number of days for drying; the length of exposure to dryness is informed by the character of the herb. While drying, the materials should be turned to expose all sides to the drying process (Calín-Sánchez *et al.,* 2020). The dried herbs should be pulverized to ensure the greatest surface area exposure when soaked in the extraction solvent. The usual primary solvents include ethanol, methanol, other organic solvents, and sometimes water (Zhang *et al.,* 2020). The problem with water is retrieving the extract from the medium, unless a freeze drying process is employed. For the other solvents, the retrieval of the extracts is facilitated by the use of rotary evaporators (Shang *et al.,* 2017). It is expected that the extract is deployed for pharmacological evaluation in this state, rather than in its natural state. It is after the extract has demonstrated potential capacity for the alleged use by the folklore, that there may be further fractionation into non-polar, intermediate polar and polar fractions. The investigation carried out with the parent extract should be repeated with the fractions, with the aim of locating the fraction with the activity detected in the parent extract (Liu, 2008). The fraction with activity may be further fractionated until a pure compound or compounds are isolated and biologically tested for activity.

Choice of doses and guiding principles

There is no one best way of choosing the very first dose when experimenting with a novel extract. Without the benefit of a beacon to reference, a recourse to some principles will suffix. A pharmacologist is interested in dose-response relationships, particularly as a basis for characterizing the novel extract (Tsatsakis *et al.,* 2018). Does it augment or reinforce the rodent behaviour, does it attenuate the response or neutral to it? The dose-response analysis answers this question. But in what format must this necessary first step be taken? We have the trial and error method of picking the first dose, whatever the method of determining the dose-response relationship. The traditional method of doing the dose-response is to try an arbitrary dose, say 0.1 mg/kg body weight, (or multiples of that i.e 1 mg/kg, 10 mg/kg, 100 mg/kg etc) and start a geometric progressive addition henceforth (i.e 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4 mg/kg etc). The geometric progression ensures that doses are not close and wastage of materials and animals are avoided (Nair & Jacob, 2016). When consecutive doses fail to elicit increased response, it should be noted that the maximum response has been attained. Further increase in dose can lead to the phenomenon of tolerance, the equivalent of tachyphylaxis in isolated tissues. From the dose-response experiment, the therapeutic index, median effective dose, maximum tolerable dose, minimum toxic dose, lethal dose (LD_{50}) and no-effect dose can be determined.

While the above holds for novel exposure of the animal to the extract, in determining the indices of sedation and evaluating anticonvulsant effects of extracts; it is different when evaluating anxiolytic effects. For anxiolytic effect, the maximum dose deployed must be lower than the minimum dose that produced sedation (Gürbulak *et al.,* 2018). The principle guiding this is that most sedatives also have anxiolytic effects because of commonality of the receptors pathway. Indeed the gabaergic/benzodiazepine receptors are mostly responsible for anxiolysis. If the animal is administered a sedative dose in an anxiolytic evaluation, the researcher will have a false positive or false negative result. In the popular model of anxiety evaluation, the elevated plus maze, the animal will be sedated in either of the arms. If the effect of the sedative becomes manifest in either of the arms, the animal will spend more time there. If in the open arm, it is assumed it is anxiolytic, if in the closed arm, it is assumed it is anxiogenic; but both are false. Recall that the frequency of entries into both open and closed arms are also essential data for establishing anxiolytic/anxiogenic effect (Vogel 2002). Sedation will also reduce these movements.

Indices to score and interpretation of results

In a comprehensive catalogue of quantifiable behaviour of rodents (Lezak *et al.,* 2017), many quantifiable indices abound. For locomotor depressions, interpreted as possible sedation, rearing, grooming, face wash, stretch attend, line crossing and wet dog shakes are indices that are quantified and the frequency must be lower than those for the control experiments (Watson & Gorzalka, 1999). Against the negative and positive controls, the frequency of these

indices is quantified and compared. Higher frequency of rearing indicates stimulation of the CNS, while lower frequency indicates sedation (Olayiwola *et al*., 2007).

In the elevated plus maze for the evaluation of anxiolytic activity of an extract, the frequency of rearing, the number of entries in both closed and open arms of the maze, as well as the cumulative time spent in each of the arms within 300 seconds recording time are noted. It is imperative that the animal keeps moving and rearing as indication of no sedation (Bixler & Ellis, 2004).

For anticonvulsant assessment, the frequency of convulsions is noted within a 60 minute period. Note that the dose of the chemoconvulsant agent employed is a dose that kills the mice after convulsion. Therefore, a successful anticonvulsant agent will be that extract which protects the animal and blocks convulsion. It is possible that the extract prevents death but the animal may convulse, here the number of convulsive episodes may be of importance (Dighe & Barve, 2019).

In the antipsychotic experiments, it is important and mandatory that an experienced assessor does the observation and recording after the researcher has administered the drugs to the animals. Because the observations are mostly subjective, bias is reduced when there is an independent but experienced assessor doing the recording of the observations (Althubaiti *et al.,* 2016). It is a parametric data generating experiment and bias must be reduced as much as possible.

Table 2: Investigations, quantifiable indices and remarks

Investigation	**Quantifiable index**	**Remark**
Sedation	Rearing frequency	<control=CNS depression
	Grooming frequency	<control=CNS depression
	Line cross frequency	<control = muscle relaxation/ CNS depression
Stimulation	Rearing frequency	>control=CNS stimulation
	Grooming frequency	>control = CNS stimulation
	Line crossing	> control = CNS stimulation/ anxiolytic?
Anticonvulsant	Frequency of convulsion	No convulsion = strong anticonvulsant Reduced frequency of convulsion without death = mild anticonvulsant Convulsed but no death by cut-off time = mild anticonvulsant
Antidepressant	Time to despair	Compared to negative and positive controls
Learning and memory	Escape time, escape route, time to recognition novel objects	Values compared to training values
Anxiety	Frequency of rearing, time spent in close and open arms of elevated plus maze, frequency of entry into both open and close arms. Droplets of urine, pellets of defecation	Experiment limited to 300 seconds; animals must perform natural behaviours to establish non sedation. Compared to control (> = anxiety)

Determination of mechanism(s) of action

This is the most important aspect of pharmacological experimentation, coming next in importance after the establishment of safety of the extract in animals. In other words, after establishment of the safety and pharmacological activity of the extract, the mechanism of action is imperative. Mechanism of action was taken with levity in most pharmacological reports in the past, especially in the era of isolated tissue preparations (Jespersen *et al.,* 2015).

In this pharmacological era, the use of isolated organs and tissues made definitive assessment of mechanism of action tedious, unreliable and mostly speculative (Borges, 2022). The level of scientific exposure in the 1970s and part of 80s when isolated tissue and organ preparations were employed to assess biological activity of extracts by other arms of pharmacology was not applicable to neuropharmacology (EFSA, 2020). The use of intact and living animals are needed in neuropharmacology where the behavioural changes in the animals are essential pointers to possible effects of the extract. It is these set of natural rodents behaviour that form the basis for ascertaining the mechanism of action of the extract. These actions or behaviours are governed by known receptors. These receptors are then potentiated or blocked by appropriate chemical agents that may be agonists or antagonists of the receptors. Here, the knowledge of the pharmacology of the behaviour is required. A mentorship program, formal study and reading of neuropharmacological topics in journals will help. For example, muscimol will potentiate gaba receptors (Akanmu *et al*., 2005; Akk *et al.,* 2020); flumazenil will block the same receptors (Kuver & Smith, 2016). In the absence of flumazenil, one can use a strict dose of picrotocin as an antagonist (Kim & Hibbs, 2021). This is a dose of picrotocin that is non convulsive.

With sedation and the receptors responsible, it may be expedient to interrogate the muscarinic, adrenergic, serotoninergic, and gabaergic receptors, especially knowing that they are all involved in the phenomenon of locomotion (Nascimento *et al.,* 2019). Note also that the index of the assessment of sedation is the locomotion, particularly rearing, grooming, face wash and stretch attend. So, it will be expedient to see if atropine will block the muscarinic receptors; if yohimbine will block the adrenergic receptors; if clozapine will block the serotoninergic receptors and if flumazenil will block the gabaergic receptors (Bates, 2020). Anticonvulsant investigation is simpler as the convulsant agent employed also gives the neuronal pathway at the same time. Picrotocin, leptazol, bicuculine, yeast, all act via the gabaergic pathway while strychnine acts via the glycine pathway (Johnston, 2013). This is the picture for chemoconvulsive agents. For electroconvulsion, while the extract may be able to block such convulsion, it is complex trying to find out if it is glycinergic or gabaergic pathway (Mishra *et al.,* 2015).

For the antipsychotic model which utlizes amphetamine and apomorphine to induce stereotyped behaviour, the amphetamine stimulates the pre-synaptic neurons while the apomorphine stimulates the post-synaptic neurons (Dépatie & Lal, 2001; Olayiwola and Ibikunle 2013).

In the learning and memory model, scopolamine, a potent anticholinergic agent is used to obliterate the learning and memory capacity of the animal. It is a strong antagonist of the muscarinic receptor responsible for memory (Rasch & Born, 2013).

Statistical inferences

Statistics apply to all facets of the experimentation and evaluation of herbal extracts; and as common to the biological systems, statistical samples are usually kept at 5 or more (n=5), especially for the animals. The rationale for the n=5 is to ensure reproducibility of data, account for experimental errors, as only this step ensures that wherever the experiment is replicated, results will be consistent. Data are computed to obtain the standard error of means. This shows the margin of errors as well as freedom from errors. When data collation is done and the error margin, depicted as ±, is large, the step to take is to look at the skewed input and repeat the experiment to generate a consistent value. Here, the number of experiments can be increased by one or two, giving the advantage of greater n (n=6 or 7); this has the advantage of making the data more robust, while taming the error margin. In some situations where the skewness is not tamed, the additional experiments can replace the existing results, keeping n as it was (n=5). Two important issues are resolved finally by statistics: the pronouncement of dose dependence and significance of difference. The data are subjected to appropriate statistical analysis such as Student t-test or Analysis of Variance (ANOVA) to determine whether there is a significant difference between set of data. An approximate approach is the addition to or subtraction of the standard error of mean from the mean and the value must be different from the next set of results in the data for one to be significantly different (3.45±0.26; 3.96±0.25). The scenario painted in parenthesis does not depict significant difference; but it depicts a shift in the response, just that the shift is not statistically significant.

Layout of report

Reports are expected to follow an order common to most journals, but a few journals adopt a different format. The common format puts the title, author(s) name(s), affiliation(s), corresponding author and contact, abstract, introduction, methodology, results, discussion, conclusion, declarations (conflict of interest, acknowledgement, funding, authors contributions) in this sequence. The instructions to authors will spell out formats unique or peculiar to the journal. It should be appreciated that reviews also follow the above format, as the method of gathering materials and data for the review should be stated. Simple expressions in English language bring out the beauty of studies and reports. Where the language of expression in the report is not the official language of the author, the services of a language editor may be employed.

Scaling to clinical level

Animal studies are the essential starting point in the discovery of new drugs or lead molecules (Van-Norman, 2020). Until the study is escalated to the clinical level, it remains an academic exercise and research for research sake. This is not the intent of an average researcher, but the researcher is caught in an undesirable *cul de sac.* Clinical trial of a candidate drug is costly, laborious, multidisciplinary, dicey, may be unrewarding, and time consuming. It is not within the purview of only one professional group to take drug from discovery to the shelf; all branches of science are involved, including social scientists. However the clinicians take a lead in the clinical trial of drugs. A paradox appears in the horizon here, as most clinicians have limited faith in herbal remedies. A few of the clinicians who approve of herbs will like issues of standardization resolved. A lot of herb-drugs interactions remain unresolved, making safety pronouncement equivocal (Pan *et al.,* 2022). An enterprise that saw theophylline, digoxin, vincristine and atropine deployed successfully without synthetic option; almost remained frozen in terms of development. Why the developmental freeze? Why is herbal research terminating at the preclinical phase and not progressing to the clinical investigations phase? What has happened to the hyped breakthrough announcements in the past with some herbs? Indeed why is the internet awashed with empirical formulations that do not work as advertised? Why has the issue of safety and standardization of herbal preparations slipped into the *laissez-faire* status?

Conclusion

This review was conceptualized to assist graduate students who did not have a good grounding in animal experimentation. I hope this has been largely achieved. The review was also premised on experiments that we routinely perform in our laboratories, making the teaching of it flow from experience largely. The review has looked at what most researchers do rightly without explanations, and those things that they do wrongly without knowing the basis. Several models and the governing principles have been elucidated, making it easier for researchers to navigate the rest of the way easily. Adequate references have been provided to see works done on the models. The critical aim of the review is to show that both synthetic drugs and herbal remedies can be evaluated using the same models and applying the same yardstick for the pronouncement of their safety. Because it grows in your garden does not make it safe, perform the safety checks on the extracts. For those who claim to inherit the knowledge of herbs from their parents, please revalidate from those who know. You would have seen from this review that herbal remedies are more involving than just empirical claims. Kidneys, the most susceptible organ of insult from herbal abuse, are mostly irreversibly damaged (or cost of restoration via multiple dialysis is prohibitive). When the sweetness of free or cheap herbs has disappeared, the agony of not discerning the source of your herbal remedies will linger.

It is very important that toxicological studies that create the gap in the confidence and acceptability of herbal remedies be escalated so that the herbs can progress into clinical trials.

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