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EFFECT OF DRUGS;

Bahavior of mice measured by modified hole board head-dipping exploratory test parameter.

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ABSTRACT... Objective: The aim of this study was to determine head-dipping exploratory test parameter as a measure of strong modulating effect on brain and behavior. **Design:** It was an observational animal study. **Setting:** University of Karachi. **Period:** Jan 2004 to July 2006. **Material & methods:** In this present study, drugs used reserpine, nux- vomica; anacardium and chlorpromazine were wide range of pharmacological actions. We evaluate the effectiveness of these drugs as agents with modulating effect on brain and behavior accessed by head dipping parameter. In this study, 25 mice were included belonging to both sexes. The study animals were divided into five groups of five animals each. Four groups were given drugs and one group was kept as control. Mice (20-35g) of either sex were used in this study. One group was kept as control for drugs. Mice were kept under room temperature. Tap-water was allowed ad-Libitum.30 minutes after giving drugs, animals were observed for 10 minutes with two minutes of interval. Tablet crushed in 10ml of water, 1cc was given. Screening method used was head dipping. **Results:** Strychnos Nux-Vomica when used in a dose of 0.07mg has strong action on cholinergic system, CNS activity and frequent head dipping (39.8±28.8) was observed. Rauwolfia serpentine is an active alkaloid particularly present in reserpine (62.2±43.4) no significant head dipping effect was observed. Anacardium (37.2±28.6) & Chlorpromazine (39.4±32.4), show decrease effects. Keeping in view, the medicinal importance of these herbs, our present study was designed to screen these drugs for CNS activity on albino mice.

Key words: Exploration, Hole-board, Head-dipping, Mouse Behavior.

Article Citation

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INTRODUCTION

Head-dipping is a measure which reflects activity and exploration. When faced with an unfamiliar environment or object, animals often exhibit behavior patterns that broadly can be termed exploration, such as locomotion around the environment, orientating towards novelty, and touching or sniffing novel objects. Exploration potentially provides an animal with new information about food sources, shelters or mating opportunities. However, by entering a new environment or attending to a novel stimulus, an animal might also increase its risk of predation, aggression from conspecifics or other hazards. Whether an animal investigates or avoids novelty has been described as the outcome of an approach–avoidance conflict or as a balance between neophilic and neophobic tendencies. In motivational terms, neophilia can be defined as the attraction that an animal displays towards an object or place simply because it is novel, while neophobia is the aversion that an animal shows towards approaching a novel object or place. In behavioural terms, neophilia and neophobia can be considered respectively as curiosity-based approach to and fear-based avoidance of a novel stimulus[1,2].

In 1964, hole-board apparatus first introduced by Boissier & Simon[3]. Since then, it has been extensively used to study drug effects by head-dipping test to examine the behavioral changes produced by a variety of drugs. Its application as a screening method has narrow spectrum because of its failure to distinguish between drugs those which are divergent in their effects. This head-dipping apparatus is a horizontal board perforated with evenly-spaced holes. A 'dip' is scored when mice lower their head far enough into a hole for the eyes to disappear beneath the plane of the board. The test situation has been altered by dividing the board with low, narrow floor into square compartments (10 x 10 cm), each with a hole (3, 5 cm

diameter) at the centre, so that additional behavioral parameters can be observed. Thus 'rearing' is scored when mice raise themselves onto the hind legs and the fore-paws rest on a partition wall and 'crossing' is scored when the animal climbs over the wall dividing one compartment from another[4].

Stress plays the main role in the pathogenesis of mental disorders. Anxiety and depression are extremely common, dramatic and debilitating multifacetic disorders, and it is now becoming clear that without knowledge of both clinical and biological aspects of anxiety and depression, it is impossible to offer effective treatment strategies for the patients. [4] We use animal model as "experimental preparations developed in one species for the purposes of studying phenomena occurring in another species". Mice and humans share more then 90% of their genes, and animal models seem to be a useful tool in medical sciences, as evidenced by a notable increase in the number of active laboratories working in this field. Furthermore, animal models are particularly of help in situations when the impact of stress cannot be studied in humans because of ethical reasons.

However, the choice of which biological correlates to study is not easy, since problems with animal models of human psychic disorders include: (i) the difference between human's and non-human's nervous systems; (ii) the difficulty in determining analogous behaviors among species; and (iii) the need in extrapolation of results from animals to humans. Such problems most likely reflect a significant difference in etiology and complexity of anxious or depressive behaviors. In addition, it is important to know that the data derived from animal models are of value only to extend that the models are valid, and that the level (severity) of the disorder evoked in animals may not be the level of human disorder we want to model. One of the current challenges is therefore to utilize the best of both clinical and neuro-pharmacological approaches to brain and behavior[5,6,7].

To study the effect of drugs on the brain and behavior, particularly in the context of searching and developing new drugs/ treatments for mental disorders, major psychopharmacological advances in the 20th century for example the development of tranquilizers, antidepressants, lithium carbonate (for bipolar disorder), certain stimulants (including amphetamines), and antipsychotic agents such as chlorpromazine (Largectil), fluphenazine (Prolixin), and haloperidol (Serenace) has been established. Psychopharmacology, a discipline that merges the subject matter of psychology, which studies cognition, emotion, and behavior, and pharmacology which characterizes different drugs. Thus, psychopharmacology focuses on characterizing drugs that affect thinking, feeling, and action. Psychoactive drugs may originate from natural sources such as plants and animals, or from artificial sources such as chemical synthesis in the laboratories. These drugs interact with particular target sites or receptors found in the nervous system to induce widespread changes in physiological or psychological functions. Clinical studies are very specific, typically beginning with animal testing, and ending with human testing[8].

Products based or herbal medicines are one of the major frontiers of research. The apparent simplicity of herbal-product after experimental studies proved to be deceptive. Central nervous system effects have been studied more extensively because of its easy availability and its involvement in many symptoms such as depression catalepsy, convulsions and coma etc. Many new structural-analogues are being developed in the management of psychiatric illnesses and effect memory. In our study, the effects of some herbal medicine were observed for psychopharmacological profile. Chemical investigation of the herbal drugs help us in exploring another use of these herbs / alkaloids and / or better understanding of adverse effects that could be seen by the use of these herbal-products[9]. Nux-Vomica is the dried ripe seed of strychnos nux-vomica Linne belongs to family-

Loganiaceae. Strychnos is the Greek name for a number of poisonous plants. Nux-vomica derived from two Latin words that mean a nut that causes vomiting. Nux-vomica tree is about 12 meters tall, grows in Sri Lanka, India and North Australia.

The seeds bark and leaves of strychnos nux-vomica contain strychnine, a highly poisonous substance that seriously damages the nervous system. Strychnine in minute doses has a beneficial effect on body, supporting the digestive system and improves urination. In high doses, strychnine is extremely toxic as a CNS stimulant[10,11]. The alkaloid produces excitation of all parts of the CNS and blocks inhibitory spinal impulses at the postsynaptic level. This results in toxic convulsions. Brucine is less toxic than strychnine and is used commercially as an alcohol denaturant[12]. Reserpine, alkaloid isolated from the root of the snakeroot plant (Rauwolfia serpentina is a snake root plant belongs to the Apocynaceae.), a small evergreen climbing shrub of the dogbane family native to the Indian subcontinent.

Extracts of rauwolfia-serpentina have been used in the study. Primarily, as Ayuvadic medicine for a variety of conditions including snakebite, hypertension, insomnia and insanity. The active constituents of rauwolfia-serpentina are indole alkaloids such as reserpme, rescmnamme, yohimbine, ajmaline and serpentine. In 1940, Indian physician had recognized two distinct properties of rauwolfia, one as a hypotensive effect and other as a sedative effect. They began using the agent for clinical-purpose[13,14].

After the isolation of reserpine in 1952, it was used to lower high blood-pressure, and its property of producing severe sedation as a side-effect also made it useful in psychiatry to use it as a tranquilizer in the control of agitated psychotic-patients. Reserpine produces its antihypertensive effects through depletion of catecholamine (adrenaline and noradrenalin) from peripheral sites. The hypotensive effect is mainly due to a reduction in cardiac output and peripheral resistance. Large doses causes hypothermia and respiratory depression. The cardiovascular effect of reserpine includes hypotension, reduced heart rate and cardiac output. The hypotensive response of the drug is due to impairment of adrenergic-transmission results in increased parasympathomimetic effects including increased gastric acid secretion, G.I hypermotility and miosis[15,16]. Anacardium is the marking nut of the Semecarpus Anacardium, a small tree belonging to the Anacardiaceae. A tincture is prepared from the crushed seeds (marking nut). The anacardium patients suffer from a very peculiar and contradictory state of mind such as laughing at serious matters and serious over trifling things. They also suffer from fixed ideas as their mind and body is separate; they suspect everybody and everything around them. They are also subject to illusions of hearing and smell. Anacardium patients have a peculiar sensation of a hook or a pin on the surface of the body as also a sensation of a plug causing a pressing penetrating pain. These sensations whenever present and in whatever ailment will make it a first rare, remedy.

Chlorpromazine, one of a group of tranquilizing drugs called phenothiazines that are useful in halting psychotic episodes. Phenothiazines have anticholinergic activity. Chlorpromazine, sold under the trade name Largactil, is often used to reduce the severe anxiety and agitation and the overactivity. Largactil was the first to be widely applied to mental disorders and remains one of the standard drugs. Drugs of the phenothiazine family are most useful in the treatment of schizophrenia. They are thought to act in part by blocking dopamine receptors at the synapse, reducing brain activity. The phenothiazines and clozapine have been credited with a revolutionary transformation of mental health care, enabling increasing numbers of psychotic persons to function outside the hospital. Antipsychotic drugs may have negative side effects, such as the dulling of physical

and mental functioning, tardive dyskinesia, and sedation. Chlorpromazine induces muscle relaxation and attenuate schizophrenic catatonia. Chlorpromazine lowers seizure threshold. Phenothiazines block alpha-adrenergic receptors and cause hypotension and failure of ejaculation. Chlorpromazine exhibits a quinidine like effect on cardiac potential. So it can cause bradyarrhythmias. Chlorpromazine inhibits the secretion of ACTH, growth hormone, gonadotropins, ADH and insulin (neuroendocrine blocking effect)[17,18]. Chlorpromazine is a classical neuroleptic. It acts on particular areas of brain to decrease dopaminergic neuronal firing. It is used as a standard psychotropic[19].

MATERIALS AND METHODS

In this study, 25 mice were included belonging to both sexes. The study animals were divided into five groups of five animals each. Four groups were given drugs and one group was kept as control.

Type of study

It was an observational animal study approved by University of Karachi, research ethics committee. Different neurobehavioral parameters like head dipping, latency number, defecations, urinations, total distance traveled/squares crossed, distance/squares crossed in the inner area, distance in the outer area, self-grooming were monitored. During each 10-min trial, behavioral data were recorded onto a spread sheet that was divided into 60- 120-s time blocks.

a) Subjects and housing

We selected healthy mice from animal house of Faculty of Pharmacy, University of Karachi. Doses were given to them for 21 days. After 21 days of dosing, the activity was seen on different models. The animals of this experiment were male and female mice. The animals were housed in a single room, which was controlled for temperature and humidity and was maintained on, a 12-h light: dark cycle (lights on at 06:00pm). The animals were housed in same-sex pairs in plastic and wire mesh home-cages (measuring 25 cm × 45 cm × 15 cm) with ad libitum access and water.

b) Apparatus and experimental design

The hole-board apparatus consisted of a wooden, white box, measuring 68 cm × 68 cm. The walls were 12 cm high. Four holes (2 cm in diameter) were cut into the floor of the apparatus; each hole was 12 cm from a corner of the box along the diagonal from the corner to the centre. The floor of the box was marked out into four outer areas and one central area using black masking tape. The central area was delineated by four lines of tape each 20 cm from one of the walls, while the four outer areas were marked out by diagonal lines of tape running from the corners of the floor to the corners of the central square. The four holes were thus located at the corners of the central square. The apparatus was located in a small testing room with dimmed white lighting. The stand of the apparatus was open on all sides, allowing the floor or objects to be dimly lit. Each subject was tested ten times in the hole-board apparatus, once per day during two sets of five consecutive days. All trials were carried out between 09:00am and 1:00 pm., and trials on males and females were alternated throughout the day. Each trial lasted 10 min with two minutes of interval. At the end of the trial, the subject was immediately placed into a carrying box and returned to the home cage. Between each trial, the floor and walls of the apparatus were cleaned with 70% alcohol solution.

c) Behavioral measurements

During each 10-min trial, behavioural data were recorded onto a spreadsheet that was divided into 60-120-s time blocks. The following behavior pattern was recorded:

Head-dip: the animal places its head into one of the holes, to a minimum depth such that the ears were level with the floor of the apparatus (a new bout of head-dipping was recorded if the animal raised its

head fully out of the hole before resuming).

OBSERVATIONS AND RESULTS

All mice were weight between 20 to 35gms. Our final analysis applied to all 25 mice that completed the study protocol. The values of the parameter was compared and analyzed by student t- test version for statistical significance. Statistical analysis using the student t-test revealed non- significant distribution for the behavioral data. The number of parameters was analysed by using student t-test followed by if there was a significant analysis to detect statistically significant differences between the groups. The level of significance was set at $P<0.05$ & $P<0.1$. All values were expressed as mean±SEM.

STUDY ON MICE

30 minutes after giving drug, animals were observed for 10 minutes with 2 minutes of interval. Tablets crushed in 10ml of water, 1cc was given. Screening methods used was Head dip. Mice (20-35g) of either

Drug	Dosage
Reserpine	0.06 mg
Nux-Vomica	0.07 mg
Anacardium	0.08 mg
Chlorpromazine	100 mg / 60 kg

Table-1.1. Drugs with dosage

Drug	Dosing (mg)				
	1	2	3	4	5
Control	-	-	-	-	-
Nux-vomica	0.06	0.06	0.07	0.08	0.09
Reserpine	0.06	0.06	0.07	0.08	0.09
Anacardium	0.06	0.06	0.07	0.08	0.09
Chlorpromazine	0.06	0.06	0.07	0.08	0.09

Table-1.2. Table of dose pattern given to the mice (n=25)

Drug	0 min	02 min	04 min	06 min	08 min	10 min	Mean ± SEM
Control	-	27	24	18	16	12	**16.16±6.50
Reserpine	-	23	19	16	11	03	*12.0±6.57
Nux-vomica	-	04	06	10	12	13	*12.66±4.7
Anacardium	-	16	11	06	03	01	*6.16±4.4
Chlorpromazine	-	14	10	07	04	02	*6.16±3.7

*Values are mean ± S.E.M. (n=5) non- significant differences by student t-test * P<0.05, **P<0.1 as compared to control.*

Table-1.3. Effect of drugs on hole board test (Mean ± SEM) (n=25).

Drugs	Head Dip	Latency number	Defecations	Urinations
Control	↓↓	No significant effect	No significant effect	No significant effect
Nux-vomica	↑↑	↑↑↑	↓↓	↑↑
Reserpine	No significant effect	↓↓	↓↓	↑↑
Anacardium	↓↓	↓↓	↓↓	↓↓
Chlorpromazine	↓↓	↓↓	↓↓	↑↑

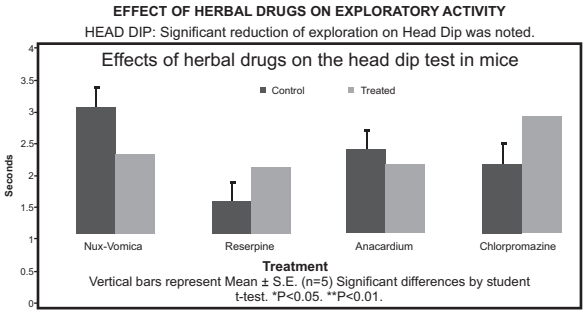
Table-1.4. Table of significant and non significant effect of drugs on head dip, latency number, defecations & urinations (n=25)

sex were used in this study. One group was kept as control for drugs. Mice were kept under room temperature. Tap-water was allowed ad-Libitum. Following drugs and corresponding doses were used:
In head dip test, mice were treated with different drugs. It was observed that there was a significant increase in head dip responses in mice when treated with nux-vomica at doses of 0.06 ug/g - 0.09 ug /g as compared to control. The effects are given in table 1.3.

Treatment	Head Dip
Control	70. ± 6.35
Nux-vomica	*62.2 ± 43.4
Reserpine	*39.8 ± 28.8
Anacardium	*37.2 ± 28.6
Chlorpromazine	39.4 ± 32.4

*Values are mean ± S.E.M. (n=5) non- significant differences by student t-test * P<0.05, **P<0.1 as compared to control.*

Table-1.5. Effect of drugs on head dipping exploratory activity of mices (n=25)



DISCUSSION

The effect of exposing mice to a hole-board in reducing the number of head dips made on a subsequent exposure was studied. The period of first exposure that was effective in causing this reduction, the interval between exposure that must elapse before this effect appears and the duration of the effect were also determined. Behavioral repertoire of animals has long been used to detect effects on, and impact of, brain and behavior. A number of models, based on animal emotional reactivity, have been designed and proven to be bidirectionally sensitive to stressful manipulations, including those of anxiety and depression. Many of these models have been successfully used to test new anxiolytic or antidepressant drugs to understand the underlying neural mechanisms by simple, rapid and inexpensive ways of evaluating an animal's condition. Although a substantial progress has been needed to make in our understanding which stressors may affect behavior and how, there are several key questions in this field which still remain open. Can we distinguish between human & animal anxiety and depression? Do we have reliable neuropharmacological tools to assess anxiety and depression in human & animals? Do we always provide correct interpretations of behavioral changes seen in experiments? Since classification of experimental animal anxiety and depression is as difficult as classification of human anxiety and depression spectrum disorders. So, the main task is therefore to differentiate between common and specific stress-related pathogenic mechanisms of the disorders belonging to this spectrum. Animal anxiety and depression taxonomy can be based on the nature and type of stressors employed, with the continuum of animal models used in experimental research ranging from "basic" animal assays to sophisticated homologous models[20,21].

Animals when exposed to an unfamiliar environment or object, often exhibit behavior patterns that broadly can be termed exploration, such as locomoting around the environment, orientating towards novelty, and touching or sniffing novel objects. Exploration potentially provides an animal with new information about food sources, shelters or mating opportunities. However, by entering a new environment or attending to a novel stimulus, an animal might also increase its risk of predation, aggression from nonspecific or other hazards. Whether an animal investigates or avoids novelty has been described as the outcome of an

approach–avoidance conflict or as a balance between neophilic and neophobic tendencies. As described by[22] those in motivational terms, neophilia can be defined as the attraction that an animal displays towards an object or place simply because it is novel, while neophobia is the aversion that an animal shows towards approaching a novel object or place. In behavioral terms, neophilia and neophobia can be considered respectively as curiosity-based approach to, and fear-based avoidance of, a novel stimulus[23]. The exploratory behavior of rodents has gained recent interest within a number of areas of behavioral pharmacology. However, Renner in 1990[24], indicated that considerable controversy still surrounds the question of how best to measure exploratory responses in laboratory animals? However, some researchers have argued that forcing an animal to be in an enclosed area, or on an open platform, does not allow the animal to exhibit its 'motivation' to explore an unknown environment, as the task evokes a strong fear response. Corticosterone levels have been found to rise in rodents on exposure to a novel open field environment, and open field behavior is influenced by some anxiolytic (anxiety-reducing) substances. Two recent studies on hole board test have also provided evidence that head-dipping by mice decreases on repeated exposure to the hole-board apparatus. If this behaviour is a valid measure of neophilia, head-dipping is also predicted to be higher in the presence, than in the absence, of objects[25].

In our study, the results show that head-dipping was high during the first test, decreased over following the trial and remained relatively stable during the rest of the experiment. Our result is in accordance with the study by Prut & Belzung, 2003[26]. Initial drop in head-dipping following the first trial could be interpreted in two ways. First, head-dipping could be indicative of a neophilic response that decreases as the animal becomes familiar with the apparatus, i.e. head-dipping represents directed exploratory behaviour that drops as the apparatus loses its novelty. If this interpretation is correct, we would also predict that head-dipping would be greater in the presence of objects; however, there was no evidence of an increase in head-dipping behaviour when objects were present underneath the holes. These results do not support the hypothesis that head-dipping is a valid measure of neophilia. The second interpretation of the initial drop in head-dipping frequency is that head-dipping could represent a fearful, neophobic response, such that, on first exposure to the apparatus, the animal actively attempts to find an escape route. Adult male mice have been shown to exhibit an increase in circulating corticosteroid levels following a single exposure to the hole-board apparatus[27], suggesting that testing in this apparatus is a stressful event. If this interpretation of head-dipping is correct, we would also predict that, as head-dipping behaviour declines, fearfulness would also decline. In favor of this interpretation, while head-dipping frequency declined over the first few tests, the amount of locomotion into the hole-board, and the time spent in it, greatly increased over these trials. Therefore, as fearfulness apparently decreased, head-dipping also decreased. If we assume that the fear experienced on exposure to a novel apparatus can be equated to normal or 'state' anxiety[28], these results contradict the assumption that head-dipping behaviour is suppressed by an anxiety-like response, in which case we might have expected head-dipping to vary in the opposite direction to anxiety-like behaviour. In a recent study by[29] review has suggested that the effects of anxiolytic compounds on head-dipping behaviour are generally confounded by changes in overall locomotion, despite the claims that head-dipping is unrelated to locomotor activity. In our study, head-dipping did decrease in frequency towards the end of the experiment, after eight or more exposures to the apparatus. Therefore, we cannot reject the possibility that, as the subjects became very familiar with the apparatus, they engaged in a greater level of visual exploration through the holes.

The exploratory behavior of laboratory animals is of

interest within a number of areas of behavioral pharmacology. However, how best to measure exploratory behavior in mice & rats remains a contentious issue. Many unconditioned tests, such as the open field, potentially confound general locomotor activity with exploration. The hole-board apparatus appears to avoid this confound, as head-dipping into holes in the floor is assumed to be a valid measure of the subject's attraction towards novelty. This study is aimed to evaluate behavioral changes produced by some herbs that are at last being scientifically investigated. There are two main focuses of this research. One is the examination and other was the evaluation of the effectiveness of some herbal extracts by using head dip assessment tool for brain and behavior. This research provides a scientific basis for the comparison of herbal remedies. The other direction of research is the search for the newer drugs among known plants or in new plant species. To establish herbalsim on scientific grounds, Psychopharmacological screening must be carried out.

Psychopharmacological screening generally indicates simply the presence or absence of a response. Thus, the fundamental elements of a drug discovery program are the bioassays used to detect substances with biological activities[30,31,32]. The CNS screening for behavior assessment includes different parameters like head dipping, open field activity, Cage crossing, Swimming induced depression & Radial-maze. There was a group in which 6 mice per group and one group was kept as control. They were provided with food and water ad-libitum and different CNS screening tests were performed. During the course of present study, the herbs Rauwolfia-Serpentina, Nux-Vomica and Anacardium were studied. Pharmacological screening of Reserpine, Nux-Vomica, Chlorpromazine and Anacardium was carried out. To determine whether head-dipping could be validated as a measure of exploration, two criteria were proposed: firstly, that it should reflect novel aspects of the environment; secondly, that exposure to the hole-board should result in information storage. Head-dipping reflected novelty was indicated by the longer duration of head-dips on initial exposure than on second exposure, the shorter the duration of head-dips. Information storage was indicated by habituation on re-exposure to the hole-board. This significant positive correlation between head dipping hole-boards was obtained from mice. This provided some indirect evidence that mice head-dipping in the "hole-board" also reflects exploration[33]. Nux- vomica, reserpine, anacardium and Chlorpromazine were tested in the hole-board. Anacardium and chlorpromazine decreased and Nux-vomica increased the frequency and duration of head-dips. Screening of herbal medicine as psychotropic drugs has strong modulating effect on brain and behavior.

Approximately 70% of Pakistani population lives in rural areas where modern medicines are not easily available, so they rely on traditional herbal medicines for relief. This signifies the importance of Eastern system of medicine in our society and stresses the need of research on herbal medicines like some of the herbal drugs Reserpine, Nux- Vomica, Anacardium and Chlorpromazine with a wide range of pharmacological actions.

In our present study, we evaluate the effectiveness of these drugs as Psychotropic agents and accessed by head dip-parameter. Among these, Strychnos Nux-Vomica has strong action on cholinergic system. CNS activity is observed as a fact finding behavior. Rauwolfia serpentine is an active alkaloid particularly present in reserpine are used to treat essential hypertension and in certain neuropsychiatry disorders. It has sedative and tranquilizing effects, as it depletes catecholamine from the central nervous system. Rauwolfia-Serpentina has been used since centuries in folk medicine in East India. Reserpine is now used as antihypertensive and tranquilizer in western medicine[34]. Chronic reserpine treatment showed a non significant effect on water intake.

Previously, it was reported that reserpine increased water intake in the light phase and the animal consumed less water in the dark phase. Other herbal drugs such as Nux–vomica and Anacardium did not produce remarkable effect. Keeping in view, the medicinal importance of these herbs, our present study was designed to screen these herbs as for CNS activity on mice[35,36].

This cannot have been due to a non-specific rise in arousal and activity due to the presence of the drug given, because when only one drug was introduced, the animals spent shorter or longer duration looking down this hole than the others. The duration of head-dips increased and decreased at the different holes. Thus the animals' head-dipping behavior reflected the attention paid to the drug given. It might be argued that the change in environment should have elicited increased or decreased head-dipping. However, the animals showed habituation under this condition. It could be that having explored, one head-dip was sufficient to gain the information. Thus, exploration results in information storage, has been satisfied. Although it is considered that these are necessary conditions for demonstrating exploration, it is recognized that they are probably not sufficient. However, within these limitations, the results suggest that head-dipping does reflect exploratory behavior. Motor activity is involved in the measurement of head dipping only to the extent that the animal has to move to reach the holes, but it can do so slowly or with ataxia. The advantage of the measure is that it is not itself dependent on locomotion. The results suggest that duration of head-dips is a better reflection of exploration than frequency. All previous work on the hole-board has used a frequency /measure, and it would in fact be very difficult to measure duration on the "hole-board" due to the high proportion of very brief dips which occur. As activity correlated well in the hole-boards for mice, it could be that mouse head-dipping in is simply a good measure of motor activity[37,38]. Limitation of the study is that the hole-board apparatus is unsuitable for some reasons: firstly, there were too many holes for the animals to be able to discriminate between them; secondly, the density of holes is such that an animal could not display motor activity without coming into contact with a hole; and lastly, there is insufficient room to place objects under discrete holes and it is the purpose of this study to determine whether empirical evidence can be obtained which validates it as a measure of exploration

CONCLUSIONS

The exploratory behavior of laboratory rodents is of interest within a number of areas of behavioral pharmacology. However, how best to measure exploratory behavior in rodents remains a contentious issue. Evidence-based strategies for reducing excessive psychopharmacological problems should widely be implemented. On the basis of the behavioral observations for which study that have been conducted, we say with confidence that all study animals meet the standard criteria for psychopharmacological behavioral assessments during the study period. Psychopharmacological problems usually require continuous care and monitoring. Education focusing on knowledge of the disease, health, results in better care. The findings and conclusions in this study are those of the authors do not necessarily represent the official position. Drug-associated psychopharmacological problems were not associated with increased mortality risk in patients. The association between spontaneous psychopharmacology and mortality was eliminated after adjustment for co morbidities, suggesting that psychopharmacology may be a marker of disease burden rather than a direct cause of death. Thus, in the present investigation, it could be suggested that these drugs has been shown potent neuropharmacological and diuretic activities etc. These facts indicate the scientific basis of these drugs being used as a traditional medicine. However, further experiments may help to determine the pharmaceutical

potentialities of the plants as a medicine.

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