Physiological and Pharmacological study on Buprofezin neurotoxicity and its Reversal by Atropine in Laboratory Rats

A thesis submitted in partial fulfillment of the requirements for the degree of master of philosophy

In

PHYSIOLOGY

By

MUHAMMAD ASLAM

Department of Animal Sciences
Faculty of Biological Sciences
Quaid-I-Azam University
Islamabad

2019

CERTIFICATE

This dissertation "Physiological and Pharmacological Study on Buprofezin Neurotoxicityand its Reversal by Atropine in Laboratory Rats" submitted by **Mr. Muhammad Aslam**, is accepted in its present form by the Department of Animal Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in Physiology.

Supervisor:

Prof. Dr. Irfan Zia Qureshi

External Examiner:

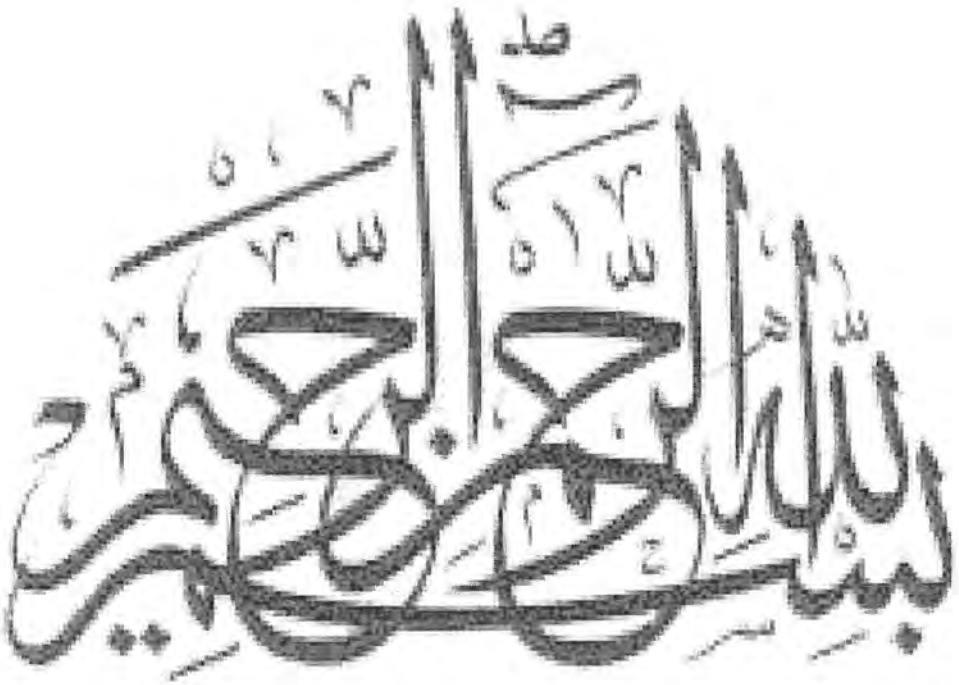
Dr. Amjad Ali
Senior Scientific Officer
Nutrition Division, NIH,
Islamabad

Prof. Dr. Sajid Malik
Chairman

Chairman
Dept. of Animal Sciences
Quaid-i-Azam University
Islamabad

Date: 13.11.2019

DEPARTMENT OF ANIMAL SCIENCES
ISLAMABAD
Date 13-11-2019
Quaid-i-Azam University, Islamabad



IN THE NAME OF ALLAH, THE MOST MERCIFUL THE MOST BENEFICENT AND THE MOST COMPASSIONATE

Dedicated to:

My Respected Parents

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously presented for any other degree.

MUHAMMAD ASLAM

ACKNOWLEDGMENT

Praise is to Allah, the Cherisher and Sustainer of the worlds, who show his countless blessings upon us. I feel actuate within myself to offer my humblest and sincerest thanks to Almighty Allah, who enabled me to compile this thesis successfully and always helped me in the ups and downs of life. All respect goes to the Holy Prophet Hazrat Muhammad (PBUH) whose teachings strengthened our faith in Allah, who is the source of inspiration to the entire humanity.

Without the support, guidance and help of many people, this study would not have been completed. I would like to extend my sincere gratitude to all those people who made this thesis possible.

I wish to express my sincere gratitude to my supervisor and favourite teacher **Prof. Dr. Irfan Zia Qureshi** for his kindness, guidance and motivation throughout my research despite his academic and professional commitments. His wisdom, Knowledge and commitment to higher standards inspired me.

Thanks to my seniors Ayesha, Zainab and 'Noreen who supported me a lot, juniors Nizam-u-din, and Khadija for their enjoyable company, my class fellows Saqlain, Nayab and sulaman for their help to overcome my difficulties. A very special and big thanks to my senior Noreen who supported me with her valuable suggestions to go through all the hang-ups throughout my M.phil.

Words always seems too shallow whenever it comes to my family. I am very much thankful to Abbu and Ammi for everything they have done to make me successful in every sphere of life.

Lastly, I offer a big credit to all my failures which motivated me to achieve this success, and to all those people who created hurdles on my way to uplift me and all those who supported me in any respect.

List of contents

List of Figures

List of Tables

List of Abbreviation

Abbreviation	Full name
ACh	Acetylcholine
AChE	Acetylcholinesterase
APTT	Activated partial thromboplastin time
ATC	Acute dose toxicity
ATC	Acute toxic category
ATP	Adenosine triphosphate
BPFN	Buprofezin
CA	Cornu Ammonis
	Chaperone *COX17*
CNS	Central nervous system
COX17	Cytochrome C Oxidase Copper
CPF	Chlorpyrios
DNA	Deoxyribonucleic acid
DTH	Delayed type hypersensitivity
EA	Ellagic acid
EFSA	European food safety authority
FDA	Food and drug Administration
FDP	Fixed dose procedure
FDP	Fixed dose procedure
FOB	Functional observational battery
GABA	Gamma amino butyric acid
GBH	Glyphosate based herbicide
Hb	Hemoglobin
HD	Head direction

ICH	International conference on harmonization
IND	Investigational new drugs
Ip	intraperitoneal
LD50	Lethal dose 50
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MSK1	Mitogen-and stress activated protein kinase 1
MWM	Morris water maze
NCI	National cancer institute
NE	North east
NOAEL	No observed adverse effect level
NVL	National veterinary laboratory
NW	Northwest
OECD	Organization for economic co-operation and Development
OP	Organophosphate
PND	Postnatal day
PNS	Peripheral nervous system
PT	Prothrombin time
RBCs	Red blood cells
RME	Reference memory error
ROS	Reactive oxygen species
SA	Sodium arsenide
SCO1	synthesis of cytochrome c oxidase
SE	South east
SEM	Standard error of mean
SNRIs	Serotonin and norepinephrine reuptake inhibitors
SW	South west

TCA	Tricarboxylic acid cycle
TCE	Trichlorethane
TSD	Time space determinant
UDP	Up and down procedure
WBCs	White blood cells
WME	Working memory error

ABSTRACT

Buprofezin (BPFN) is a thiadiazine insecticide that inhibits chitin synthesis and the moulting in case of white flies, mealybugs and leaf hoppers. The exposed insects are unable to shed their cuticle and ultimately die as moulting ensue. Neurobehavioral toxic effects elicited by buprofezin remained unclear. Furthermore the reversal of buprofezin induced neurobehavioral toxicity by atropine was not elaborated. Thus, we explored the neurobehavioral toxic consequences of acute buprofezin exposure in adult male rats and effective reversal of these changes by pretreatment with atropine as an antidote. Acute administration of commercial buprofezin (87.9mg/kg/day through oral gavage with corn oil as vehicle) induce a wide range of neurobehavioral toxicity including damage to pyramidal cells of hippocampal CA1, CA2 and CA3,region and behavioral impairments as demonstrated through, loss of motor coordination, locomotor activity, fear loss, hearing, heat shock, sensorimotor, cognitive and spatial navigation impairment following the exposure .These neurobehavioral toxic effect of acute buprofezin exposure were significantly reversed by the 15 min pretreatment atropine antidote before the buprofezin administration. Pre-treated atropine (20mg/kg/day;i.p) attenuates the neurobehavioral toxicity induced by buprofezin in adult male rats. Acute buprofezin exposure also induce hematological parameters variation as significant increase in WBCs, lymphocytes, monocytes, red blood cell distribution width and platelets were found following the exposure however pre-treated atropine counteract the effects. It was suggested that acute buprofezin exposure elevated the acetylcholine level, by inhibiting the synthesis and release of acetylcholine esterase (AChE) in synapse. But the complete mechanisms are remained to be elucidated.

INTRODUCTION

1.1 Toxicology

Toxicology is the study of toxins, poisons, their effects and treatment. To improve therapeutic potential of existing drugs and designing new drugs, toxicological screening of various drugs and pesticides is very important. It is necessary to search novel molecules for toxicity potential in animals and for pharmacological activity stated by the (FDA) Food and Drug Administration Organization in US.

Toxicology is a worldwide renowned, journal whose publications comprises of only the utmost quality original scientific research and mechanism of toxicity related critical reviews toxicity associated with exposures to foreign chemicals. The primary purpose of the journal is to develop up-to-date understanding associated to the mechanisms of toxicity, predominantly as it relates to human health.

For calculating (NOAEL) dose, toxicity testing is important, and it is also supportive for purpose of clinical studies (Setzer and Kimmel, 2003).

1.2 History of toxicology study

Toxicity studies were historically initiated with Paracelsus (1493–1541) and uncountable particular compounds those were accountable for noticeable toxic effects against animals and plants. Paracelsus, an astrologer, Alchemist and a physician declare the beneficial and harmless properties of toxic agents and narrate the relationships between dose and response for the consequences of drugs, thus he is regarded widely as father of toxicology. He narrated that all substances are poisons, but the dose characterized a poison and remedial substances, this statement is quoted frequently. (Philip Hunter, 2008). A Spanish physician, Mathieu Orfila (1787–1853), cited as father of modern toxicology who showed the association between poisons and their biological characteristics and showed that toxic substance cause specific organ damage. In mid 1990s, methodology of toxicological research and toxicological camouflage for individual compound was developed and in the mid of 20th centuries toxicological study for environmental substances developed. (Parasuraman, 2011).

Trevan (1920) initate the use of animals in toxicity studies and reveal lethal dose for each chemical by demonstrating use of the lethal dose 50% (LD50) test. For testing

results of pharmaceutical and chemical on skin and eye, after the treatment of LD50 (1920) for testing skin and eye irritation using rabbits elaborated method by FDA scientist John Draize, which was widely credeted. Through daily dosing of mice and rats for 2 years, a test was established by National Cancer Institute (NCI) of US for analysis of carcinogenic chemicals. Thalidomide which give rise exhausting birth defects symptoms expressed by thousands of young ones were born in early 1960s. Thus, after that regulatory agencies made necessory submission of toxicological data of investigational new drugs (IND) and stress on exploration of the toxicological profiles of all pharmaceutical compounds available that are used by patient regularly. In the late 1980s (OECD) and (ICH) disclosed the guidelines for toxicological analysis of pharmaceutical compounds (Parasuraman, 2011).

1.3 Classification of toxicants.

Toxic substances can be classified on basis of chemical nature, class like exposure class and use class and mechanism of action. Toxic chemical may be occurring in soil, water, air or in food classified as exposure class. Cosmetics, phytotoxin (plant toxin), pesticides, food additives, agriculture chemicals, therapeutic drug and drugs as drugs of abuse (Cope, 2004).

1.4 Various types of toxicity.

1.4.1 Acute toxicity.

To elaborate the consequence of solo dose on individual animal models, acute studies for toxicity was performed. A rodent and one nonrodent species usually accepted in short term testing of toxicity. The chemical agent was given in variable dose concentration and their consequences are analyzed for fourteen days in acute testing of toxicology. During the time course of experiment injury and destruction caused by the specific chemical are noticed. Histological, pathological, biochemical, and morphological alterations are recorded in dead animals. Acute testing of toxicity allowed to determine 50% lethal dose (LD50) of the testing which was used as sign for acute toxicity in early ages.

LD50's assessment needs large number of animals and the destruction of animal was pronounced.

Refined methods were introduced due to these short coming like (ATC) method, (FDP) procedure and (UDP) method. For assessment of the non-noxious toxicity except from the lethal concentration, FDP method was used. Experimental animals were placed under keen observation for a specific period when testing chemical is given at fixed concentrations of 5, 50, 500, 2000 mg/kg. A constant procedure of ATC method comprises of using three animal of same sex in each step. Already identified our doses can be used ATC evaluation method but depends on Globally Harmonized Classification system test dose should be selected (Stallard and Whiteheaad, 1995).

1.4.2 Repeated dose testing for toxicity

Continuous testing for toxicity was carry out for at least 28 days. Testing chemical is given through oral route for a specific period daily and if this route is not easy then testing chemical is given parenterally at a specific time regularly. continuous testing for toxicity require rodent of age 5–6 weeks and any gender was used usually and in this testing procedure, satellite group may include. Both high-dose group and control group present in satellite group. Between the animals there must be little individual variations: ± 20% differences in the weight are acceptable. Behavioral and biochemical parameters which are baseline parameters of the animals are evaluated to calculate percentage changes. In continuous testing of toxicity studies presentation of human safety details was necessary (WHO, 2008). Where experiment was being completed for recording histological changes tissues are removed from most of the organs. If feasible on the same animals immunotoxicity studies are executed but these analysis beyond the period of 14 days are not possible. Antigen- or Mitogen- stimulated lymphocyte proliferative response, delayed type hypersensitivity (DTH), primary antibody response to T- cell dependent antigens and macrophage function such parameters in immune toxicological studies may be evaluated. Sub chronic toxicity and continuous toxicity studies are distinct from each other only by duration: more than 90 days are needed to complete sub-chronic toxicity study and in more than 28 days of duration repeated dose toxicity studies are carried out (Parasuraman, 2011).

1.4.3 Sub chronic repeated dose testing of toxicity.

For understanding the sub chronic toxicity of a testing chemical rodents and no rodent animals are used. Behavioral changes and changes in monthly cardiovascular and biochemical parameters and weekly body weight variation are examined after giving orally the test substance for 90 days. When study get accomplished study animals are

slaughter. After examination of gross pathological variation in tissues. The tissues are harvested and subjected for histological analysis. Between the animals, individual variation will be less and ± 20% variation range are allowed in weight. In the study protocol a group must be included which contain control and a highly treated testing animal known as satellite group (Muralidhara *et al.*, 2001).

1.4.4 Chronic testing of oral toxicity.

In chronic toxicological studies, minimum of two animal species i.e. one rodent and one non-rodent are used. The animals are examined frequently after administrating testing chemical over more than 90 days. In animals, long lasting effects of a testing chemical inferenced by chronic toxicity might be suggested for human protection of that tentative product. It is crucial procedure to describe chronic oral toxicity of new drugs. Between the animals, little individual variation should be there and ±20% variation range is allowed in weight. In the study protocol a group may be included having both high-dose group and control group known as satellite group. Ongoing experiment, for behavioral disparity, variation in biochemical parameters and normal physiological functions animals are examined. When experiment accomplished tissues of animals from all organs are taken and proceeded for histopathological analysis (Jaijoy *et al.*, 2010).

1.5 Hematological toxicity

Hematological toxicity is study of toxic effects of chemicals in environment and workplace, drugs and other factors like exercise, ionizing radiation and stress on blood and it's forming tissues. These principles significant here only to hematological toxicities but usually it will be stressed on issue relating preclinical safety analysis and drug development. Liver and kidney with blood and hematopoietic tissues marked as main target organs notable of scrutiny is assessment of drugs in clinical and preclinical safety. Systematical administration of compounds causes blood cell destruction and elevated mitotic rate of hematopoietic tissue due to factors like impairment of bone marrow are contributing to above target organs. In normal individual, erythrocytes, thrombocytes and leucocytes and each might be generated approximately at the rate of 1-3 millions/sec (Erslev and Lichtman, 1990). Rate might be raised up to eight-fold as in disorder such as hemolytic anemia their demand for cell become high (Jain, 1986).

Total leucocyte count, RBCs, platelets count, red blood cells distribution width, percentage, amount of hemoglobin, mean corpuscular hemoglobin(MCH).mean corpuscular hemoglobin concentration(MCHC) and mean corpuscular volume (MCV) are includes in hematology test which is always mentioned in clinical and preclinical protection. Bone marrow cytology and reticulocytes counts in toxicity and protection studies are not always advantageous as screening tests might be mention when the testing compound effect the hematopoietic system. Dried smear of blood will prepare from every animal for reticulocytes count if automated reticulocyte analyzer is not available. Unless designate appropriately by effect of testing chemical on red blood cell, leucocytes and thrombocytes, conducting of microscopic evaluation for study of bone marrow cytology or reticulocyte count is not essential only slide of bone marrow to be prepared for studying cytology of bone marrow from each animal. Absolute differential leucocyte count must be evaluated to interpret leucogram. As due pointless explanation of relative differential leucocyte count alone, total leucocyte count necessarily to be multiplied by relative differential leucocyte concentration to get significant quantitatively absolute differential leucocyte count (Zawidzka, 1990).

Laboratory hemostasis test for protection and nonclinical studies like Activated Partial Thromboplasin Time (APTT), thrombocytes count and Prothrombin Time (PT) are suggested (Vargaftig *et al.*, 1979; Zawidzka, 1990). Platelets count are always determined by automated hematology analyzer in safety and toxicity studies and with every quantitative measurement of hematological analysis is suggested. Entire blood clotting method is an inaccurate laboratory test and provide little meaningful information not suggested for hemostasis as regular testing analysis in nonclinical studies (Dodds, 1980; Theus and Zbinden, 1984). As (APTT) activated partial thromboplastin time blood clotting test need large quantity of blood and thus its use is limited in toxicity and protection studies which results no specificity of test. Using of needles and syringes blood samples should be collected by venipuncture for plasma coagulation time measurement (Dodds, 1980). In rodents few technical limitations influence recommendation of this for testing of coagulation that concern with amount of blood and site of its collection. For coagulation time measurement, blood should not be drawn from orbital venous plexus of rat because time of coagulation is mostly increased with this method (Dameron et al., 1992).

1.6 Neurobehavioral toxicology.

It was clearly established that (CNS) is essential for production of behavior.It is also clearly established that human and animal behavior was altered by a several of chemicals entities. In this article behavior measure have been recognized as essential in screening the potential toxicity of these agents on central nervous system. This recognition has open a new area of research within toxicology which has been referred by Weiss and Laties (1969) as" behavioral Toxicology" and by Zbinden (1983) as "neurobehavioral toxicology"

After the decleration of early development of this area of research to the status of subdiscipline with in field of toxicology comes from the several books, book chapters, reviews and symposia devoted to this subject (Anger and Johnson, 1985).

"Functional testing for behavioral toxicity" call was published about twenty five year ago by joseph Raffin. Later the area of behavior associated toxicology or widely, neurotoxicology has revealed exponential progress and emerged as independent areas of study under the common crown of toxicology. One consequence of this advancement was that enough research has assemble to allow the establishment of evaluating plans for behavioral toxicity.Neurobehavioral and pathology are interdependent constituents of basic research and toxicological evaluation analysis of nervous system analyzed by pharmaceutical and environmental chemicals. Although neuropathological analysis give new visualization as to neuron cell variation, physiological, behavioral procedures assess the functional outcomes of neuron communication disorder. The cardinal reasons of different variation in behavior may be known, but mostly lake understanding about direct link with particular brain pathology. In some other situations, rapidly developing transgenic mouse models are crucial source of substantial behavior expression related information and altered pathologies. Summation of all nervous system activates give rise to behavior and various behavioral aspects are evaluated in neurotoxicity testing

(Moser, 2011).

The area of neurotoxicology has developed as consequence of interconnection among pharmacology, toxicology, psychopharmacology, and experimental psychology included the structural, functional variation in nervous system as a consequence of chemical subjection or effects of external environment, and cannotation of the results and harmfulness of those variation. As the (CNS) is defiantly multifaceted body organ, constituting various types of neuronal and glial cells, central and peripheral anatomical

configuration, size and structural configuration, excitatory and inhibitory synapse various neurotransmitters and require high energy for action potential. our nervous system is metabolically and physiologically unique to various toxicants and promote the liability of compounds to act on various target sites in various ways (Moser et al. 2008).Initially variation histopathology were measured the ''gold standard'' that determine the arena of neurotoxicology, till the appreciation that the toxic compound can also affect the nervous system functionality e.g. behavior, in extreme and different ways stress the inevitability of other analysis. Broadly Behavior is the miscellaneous and the result of interconnection at many levels. Variation in nerve cell communication reflects the behavioral affects and interconnection in addition to variation in morphology that can be analyzed by the pathologist. The behavior demonstrate the interconnection and nervous system integrity, it is substantially deliberated a sensitive analyzer, and the optimum test, for the functionality of nerve cell (Kulig et al. 1996)). The word pharmacology or toxicology of (CNS) refer to higher order function of (CNS), for example perception and cognition function or electrical activity of brain it is also necessary to include the PNS and lower order function of CNS e.g. innate responses, reflexes, and behavior that are critical for exist.

1.7 Behavior testing in toxicology.

In chemical (pesticides) and pharmacology industries trial based on impact of chemical on nervous system emerged on quite parallel tracks. The systematized evaluation of drugs effect on nervous system assessment in rats somewhat recruit a range of keenly observations and manipulative computation (screening) that was demarcated as the Irwin screen (Irwin 1962, 1968). Initially Chronic toxicity investigation and behavior observations in cage were became a procedure of perceiving rapidly toxicity signs, although their application this analysis for behavior was not pervasive. (Arnold et al. 1977). After the directions of multiple experties panels (e.g., National Academy of Sciences 1975), academic scientists and government e.g (Brimblecombe 1979) (U.S. EPA) originate and published recommendation for many behavior tests, constituting array of tests slovenly depends on the Irwin screening tests, called the (FOB) functional observational battery. (Sette, 1989).

These (FOB) testing finally systemized in the U.S. EPA Human Health 870 array of Test Guidelines (U.S. EPA 1998c) and were interconnected with many other test

guidelines of (OECD; 1995, 1997). Resembling assessment perspective were approved for chemicals in the (FDA) ''Red Book'' (Sobotka et al. 1996), and behavior screening of potential drugs is now demand by the (ICH) in the recent S7A guidelines (ICH 2000).

However other series of behavior tests have been established in academic circles for used in evaluation of changes in genetically modified mice nervous system. In these studies, behavior serves as a detector for precise variation in genetic, and additionally, genetic bases of behavior cab be evaluated by transgenic testing. The profile of standard methods and screening tests are currently being used. (Van der stay and stickler 2001, 2002).However these progresses have arisen in similar with the growing use of behavior tests in toxicology and pharmacology, there has been only a little convergence. Useful neurological information can be perceived by using rat as models for precise behavioral profile and, hence, inform explanation of behavior changes. (Almeida, and Wotjak , 2006).

1.8 Behavior screening tests.

First-tier testing(screening), generally composed of simple or fast behavior screening that which indicate whether a testing chemical acts on CNS, and at what concentration, however second-tier testing constitute extra complex screening analysis that give an additional absolute explanation of the consequences and association between dose and response (Tilson , 1993).Intuitive, or also known as reflex, behaviors (e.g., sensory function, locomotion) give us a wide analysis of brain function and can be assessed at both levels of analysis. For many years the FOB (functional observational battery) achieved a wide use across numerous laboratories to distinguish the effects of different chemicals, including industrial chemicals, pesticides, organometals, water contaminants, solvents, and pharmaceuticals (Moser,2010).

The U.S. EPA FOB comprises innumerable assessments of sensory, motor, sympathetic, parasympathetic, and neurology functions. There is not a single FOB protocol as decelerated, but the guidelines define further ordinary features and screening tests that should be incorporated. During several years many protocols for behavior testing have been issued (Kulig et al., 1996), and each laboratory substantially uses its own testing protocol. The behaviors analyzed in (functional observational battery) FOB screening method may be classify by areas of brain functions, although

there is wide overlapping as many tests utilize many areas and these domains do not essentially draw to particular area of the nervous system .(McDaniel et al., 1993).

However some sympathetic and parasympathetic functions can be evaluated by observation (e.g., pupil size, lacrimation, salivation,) while others (e.g., heart rate, respiration,) may need particular instruments for analysis. Neuromuscular aptitude and integration can be assessed by multiple screening tests beginning from analysis of evaluation of muscle strength, gait and posture, to analysis of hind limb and forelimb grip strength, to observing of righting reflexes. Testing methods available for sensory response evaluation in first-tier screening involves either simple reflexes screening (e.g., pinna reflex, or grasping) or analysis of the motor response to a diversity of sensory input (e.g., somatosensory, auditory, nociceptive). Action or reaction analysis may be automatic or perceived as part of the TSD maze observations (e.g., rearing, arousal). Yet another screening battery suggested for analysis as an organized, objective method for screening mouse behavioral phenotype is entitle SHIRPA (Rogers et al., 1997). The test comprised of primary (observational battery), secondary (objective measures, e.g., analgesia, motor activity,) and tertiary (characterization studies of sensory, cognition, anxiety, electrophysiology) final results. Most of the motor activity tests e.g. grip strength and foot splay assay, are included in an FOB or the Irwin battery at screening level. Rotarod, is an automated test mostly used for motor function test, which that assess the ability of testing individual to sustain equilibrium on a rotating rod that is rotating at either a static or constantly increasing velocity (Dunham et al., 1957).

It has been extensively used to measure effects of neurotoxicants and drugs in rats and mice models (Gerald et al.,1977) and also for brain trauma (Hamm et al., 1994), and description of behavior in genetically modified mice (Crawley, 1999).

1.9 Motor activity screening.

Screening of locomotor activity is a crucial for functional analysis of neuron, demonstrating the highest of neural interconnection and used an index for several years to analyze impact of physical and chemical treatment (Tilson et al., 1984). There are many mazes available now a days, and detection systems include TSD maze (Aslam, 2018).

Motor function of Nervous system can be calculated in relations to motor activity, but other feature should be noticed, containing equilibrium, motor coordination and muscle strength. Only small number of tests have been recognized by their capability to distinguish acting chemicals via various mechanisms or with their biochemical or pathology discrepancies (Sheedy′ et al., 2008).

As, grip strength (Meyer et al. 1979) is analysis criteria for depression of CNS (Nevins et al., 1993), peripheral or central pathology (Nichols et al., 2005), malfunction of neuromuscular junction (Dean et al., 1991), and other general aspects. Exposure of different chemicals using various neurobehavioral screening tests: rigidity, motor activity, gait analysis, catalepsy, foot splay assay, and reflexive responses (Maurissen et al., 2003)

1.10 Tests for learning and memory. (Cognition test)

Various learning and memory test, which evaluate experience based behavior alteration , may be observe as development to positive outcomes in preliminary tests (ICH 2000), or as a demanded component in the U.S. EPA test recommendation for development of neurotoxicity (U.S. EPA 1998b).

Assessment of cognitive function has been a fundamental measure of psychology and psychopharmacological research, and countless methods that have been established for testing. Such pattern comprise simple or complex conditioned responses, spatial or positional navigation, and positive and negative reinforce behavior training.

Despite the availability of large options a few cognitive methods presently used in screening analysis (Lochry et al., 1994). For evaluation of cognition, training is required that ranges from daily testing for months or multiple trials within a single day. Although food or water constraint takes extra time, most of recently used methods are either shock-motivated or water-based.

A prominent Vol. 39, No. 1, 2011 FUNCTIONAL NEUROTOXICITY TESTING 39 test is simple shock avoidance conditioning test, e.g., passive avoidance that require only a few trials to establish the memory.

For spatial or positional navigation, water mazes tests are used and comprises e.g. Y, TSD, Biel, or Cincinnati maze, these need a decision point where testing animal learn which way has to be turn. (Biel et al., 1940).

Originally Morris (1981) describe a different type of maze in which the testing animal is necessary to use extra maze cues to navigate the hidden platform. Laboratories regulating safety pharmacology tests revealed that these laboratories uses cognitive tests comprises of passive avoidance tasks and simple water maze and these are the most commonly used tests (Lechery, Johnson, and Weir, 1994).

The automatic startle response is an additional part of the sensorimotor reactions analyzed in testing method. Mostly used startle response is the acoustic response, which usually calculate the strength of the motor activity subsequent exposure of auditory stimulus above threshold. Somatosensory, visual and air puff, stimuli can also be used. Magnitude and latency depends on each other and range of tests are available. With exposure of repeated stimulus the response usually habituate. The neural circuits of simple responses have been demonstrated, and there are few behavior whose physiological basis has been identified. (Yeoman's et al., 2002).

The supposed route consist of few synapses, auditory nerve, ventral cochlear nucleus, nuclei of the lateral lemniscus, reticularis pontis caudalis nucleus, and motor neuron of spinal cord. (Davis et al., 1982).

By varying the pre stimulus tone potency, auditory threshold can be accepted, which has been advantageous in identifying, e.g., midfrequency hearing loss caused by solvent treatment. (Crofton, 1990).

1.11 Hippocampus and memory.

So many of cognitive test recently used for evaluation of developmental neurotoxicity. It is necessary to understand substantial variation in tests while comparing young rodents and adults (Ehman et al., 2006). Pharmacological and lesion studies has provided extensive understanding about the central neurons necessary for cognition. For example function of hippocampus is perceived a major impact in spatial learning, and repetition learning (D'Hoogs et al., 2001). Though, mostly it is affected by complex integration among parts of limbic system and become difficult to explain. (Calton et al., 2009). Cognitive function routs developed as of the cholinergic system, though there is an interconnected impact of many neurotransmitter. (Ehman et al., 2006).Most cognitive tests used to evaluate the function of hippocampus contain radial-arm maze, spontaneous alternation, fear conditioning and Morris water maze (Gerlai, 2001).

1.12 Pesticide toxicity.

The ubiquitous application of pesticides in distinct areas has elevated the risk of environmental pollution by various xenobiotics that can be highly toxic for non-target organism containing human. Pesticides are extensively used in commercial and agriculture fields to control pest growth and household agents (EFSA, 2013; EPA, 2011a; Eurostat, 2003). the abundant of toxic pesticides have been banned in most countries, because they are classified as organic pollutant (Stockholm Convention, 2014), yet their component and metabolic residues are still found in environment and human.(Bedi et al., 2013; Dalvie et al., 2014; Mage et al., 2004; Toan et al.,2013; Weber et al., 2010). Several pesticides are constructed in order to attack on nervous system of pests, so due to the similar neurochemical transmission system these insecticides are neurotoxic to human at different doses.

The ubiquitous application of pesticides in distinct areas has elevated the risk of environmental pollution by various xenobiotics that can be highly toxic for non-target organism containing human. Potential hazards of these pesticides, involving their neurotoxic effects on development has been reported in previous finding (Eaton et al., 2008).

1.13 Buprofezin Toxicity.

The buprofezin acute toxicity was slight in earthworm and fishes, mammals and eating birds, but was highly toxic to aquatic ecosystem. BPFN get accumulated in aquatic milieu thus buprofezin exposed aquatic ecosystem was at hazard. Buprofezin chronic toxicity for aquatic organism was absent, though it was extensively used for regulation of insect growth. Buprofezin (BPFN) insecticide belong to thiadiazine class of pesticide that inhibit the molting process of different pests including white flies, mealybugs and leaf hoppers. (Liu and chen, 2000). It inhibit the chitin synthesis and showed its action as the molting process proceeded. The exposed insects are unable to shed their cuticle and ultimately died during this molting process, developed by Nihon Nohyaku in 1981(Chen et al., 2011).

Being one of the first insect's growth regulators it acts mainly against sucking insects. It is affluently applied against different homopterous pests in paddy fields and citrus groves, i.e. crops that have developed resistance against efficiency of many of the

organophosphates, hydrochlorides, carbamates and even plant constituents like pyrethroids. (De Cock et al., 1998).

Acute exposure of buprofezin (dermal, oral or through respiration) induce low toxicity in mammals oral and dermal LD50>2000mg/kg body weight.LC50 4.57mg/L air /4th and do not produce itching in skin and eyes. (EFSA, 2010)

Liver and thyroid are adversely affected by buprofezin and histopathological changes not occur in affected organism but it change clinical chemistry. Orally administration of buprofezin for two days induce micronuclei in bone marrow erythrocyte of mouse (Inagaki, 2006).BPFN devoid carcinogenicity and reproductive toxicity in rats or mice and not induce neurotoxicity in mammals (EFSA, 2008). In contrary our finding have proved that acute exposure of buprofezin induce wide range of neurobehavioral toxicity in adult Sprague dowely male rats. But the mechanism of neurotoxicity was still not elucidated. It also induce hematological, and body weight alteration.

Buprofezin acts on liver the main site of its action. Various researches suggested that BPFN accumulated in liver and consequently induce oxidative stress. Cytochrome C oxidase activity is retarded by buprofezin, which is most important cause of energy production and thus produce (ROS) (Ji et al., 2016). Buprofezin induced chronic toxicity and carcinogenicity has suggested that liver is chief toxicity target in mice and rats. A two year study in mice reveal that body weight of female and male mice decreased from week 6 (male)and week 9(female)onward at 5000ppm dose, but a little decreased at 2000 ppm dose. (Yoshida, 1990).

1.14 Buprofezin Toxicity in fishes.

A study reveals that sub lethal dose of only buprofezin (100mg/L) in Cyprinus carpio cause hazardous genotoxic, biochemical and histopathological changes in liver, gills and kidney. DNA smear formation following the buprofezin administration revealed single strand breaks. Secondary lamellae fusion, cellular hypertrophy, pyknotic nuclei, karyrrhexis, nuclear hypertrophy, renal tube degeneration and glomerular contraction are some of histopathological changes observed in liver, gills and kidney of Cyprinus carpio due to toxicity of buprofezin (Qureshi et al., 2016; ku et al.,2015). Also ROS production in zebrafish embryos after the administration nickel and buprofezin.

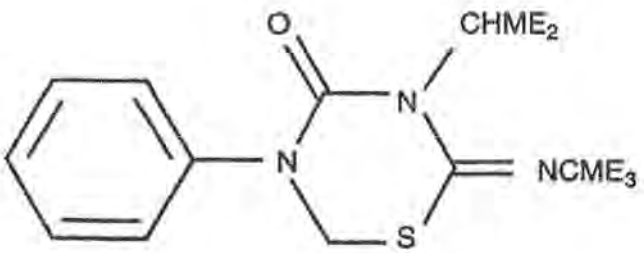
Administration of buprofezin to embryo and larva of African catifish (Clarias gariepinus) at various concentration (0, 0.05,5,25,50 and 100mg/L) cause death of embryos as the dose of buprofezin elevated from 5 to 100 mg/l.

The LC50 concentration of buprofezin for larva was 5.702(3.198-8.898) mg/L and for 24 h and for 48 h LC50 was 4.642(3.264-6.287). Even minor dose of buprofezin 5mg/L, was noxious for early developing larva and embryo of African catfish a symmetric head, pericardial hemorrhage shape, body arcuation, lordosis, yolk sac edema and tissue ulceration are the abnormalities associated with exposure of buprofezin in African catfish (Marimuthu et al., 2013).

1.15 Mode of action.

It inhibit deposition of cuticle in pests at larval stage and in it suppress egg laying in female adults by preventing synthesis of prostaglandin and alter hormone discharge that inhibit moulting in nymph (public release 2000). BPFN render incorporation of ^{3}H-glucosamin into chitin (Izawa et al., 1985). Because of chitin defciency, the elasticity of procuticle lost in whitefly nymphs and the insect was incapable to accomplish the molting. (De Cock et al., 1988).

1.16 Structure.



IUPAC name;

(Z)-2-*tert*-butylimino-3-isopropyle-5-phenyle-1,3,5-thiadiazin-4-one

I.Ishaaya *et al.* (eds.), Insecticides with novel modes of action Springer-Verlag Berlin Heidelberg 1998

1.17 Properties and metabolites of buprofezin.

In acidic and alkaline condition it is stable and gets absorbed in soil due to its hydrophobic nature. Owing to these properties buprofezin retention in soil was longer than any other pesticide (Funayama et al., 1986; Li et al., 2012). Buprofezin half -life of is about 50 -70 days, under aerobic field condition and its half -life is approximately 36-104 days in flooded field areas (public release,2000; Li et al.,2012).However buprofezin is widely used leafy crops, citrus plants and fruits crops and its metabolic remainders may cause substantial hazard to vicinage environment. (EFSA, 2007).

Soil residing buprofezin is decayed into various metabolites by soil living microorganisms. (Chen et al., 2011). Delineate transformation pathway of buprofezin via pseudomonas sp.DFS35-4 a strain that metabolize buprofezin present in polluted China. Rice field soil residing Rhodococcus sp. Strain YL-1, has capability of biodegrading buprofezin into four metabolites: 2 isothiocyanato-2-methyl-propane, 2 tert- butylimino-3-isopropyl-1, 3,5-thiadiazinan-4-one N- tert-butyl thioformimidic acid formylaminomethyl ester , and 2-isothiocyanato-propane, (Li et al.,2012).

A product of buprofezin metabolism, buprofezin sulfoxide, was readly available in the flooded soil which revealed that buprofezin could be degraded by flooded and upland soil residing microorganisms (Funayama et al., 1986).

1.18 Neurobehavioral toxicity of buprofezin its mechanism and recovery by atropine.

Acute intoxication of buprofezin induce a wide range of neurobehavioral toxicity including damage of pyramidal cells of hippocampal CA1, CA2 and CA3, neuron and behavioral impairments for example, loss of motor coordination, locomotor activity, fear loss, hearing, heat shock, sensorimotor, cognitive and spatial navigation impairment following the acute exposure in adult Sprague dowley male rats. We have also found that acute intoxication of buprofezin was potentially reversed by pre-administration of Atropine. The complete molecular and biochemical mechanism of buprofezin neurobehavioral toxicity was not elucidated so for however we have suggested that it inhibit the synthesis and release of AChE in synapse as in our experiment buprofezin intoxicated rat was suddenly dead after the administration of neostigmine (30µl/kg/day i.p) a blocker of AChE. It put forward that a small concentration of AChE in synapse was dominantly occupied by a small concentration

of neostigmine consequently tremendously elevated the ACh level in synapse leading to tremor and death of rat. This hypothesis was also supported by previous studies as activity of cytochrome and TCA cycle enzyme was rendered by buprofezin that interfere the energy metabolism and inhibit production of ATP. (Ji et al., 2016; Binukumar et al., 2010; Shan et al., 2013

With the advancing incidents of neurobehavioral and cognitive defects the objective of this study was to investigate the neurobehavioral toxicity, underlying mechanism, and possible therapeutic approach to overcome its neurotoxicity following acute exposure of buprofezin.

1.19 Atropine.

Atropine function as a physiological antagonist and competitively block the acetylcholine action of at muscarinic receptors and acts as antidote for excessive parasympathetic activation arise as consequence of inhibition of AChE. In most of extreme situation respiration is necessarily maintained by artificial ways. At the ordinarily used dose concentration, muscarinic effects is also antagonizes by atropine in the CNS, thereby partially reversing convulsions and blockade of the respiratory center. (Johnson et al., 2000).

MATERIAL AND METHODS

This study was planned, designed and conducted in physiology laboratory, Department of animal sciences, Quaid -i- Azam University, Islamabad Pakistan.

2.1 Approval of ethical committee

This study was approved from ethical committee, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad Pakistan. All of animal procedure were carried out under the standard rules established by Bioethical Committee of Biological sciences.

2.2 Male rats

For this study Sprague dawley adult male rats weighing 200-240 gm were provided by (NIH) and were accommodated in Primate Facility department of animal sciences Quaid -I- Azam university Islamabad. Rats were placed in steel cages in a room with an environmental temperature of 25◦c and 10/14 h light and dark period, with free availability of rodent diet and tap water.

2.3 Experimental design

The rats were separated arbitrarily and divided into groups. First group was taken as control second group was treated with buprofezin 87.9mg/kg/day through oral gavage with corn oil for seven days. Third group was pretreated with atropine 20mg/kg/day given intraperiotoneal (i.p) and after fifteen minutes followed by administration of buprofezin 87.9mg/kg/day for seven days. All of rats underwent behavioral test at 7^{th} day of treatment.

2.4 Chemical

Commercial formulation of buprofezin (Robon 250g/kg 25%w/w) linear formula C16H23N2OS and molecular weight 305.44g/mol CAS NO. 69327-76-0 were purchased from Jaffer group of companies. buprofezin is a 2-(tert-butylimino)-5-phenyl-3-(propan-2-yl)-1, 3, 5-thiadiazinan-4-one in which the C=N double bond contain Z conformation. It is a member of homopteran inhibitor of chitin biosynthesis works as an insecticide.

2.5 Blood profile on hematology analyzer

For complete blood picture, 2 ml of blood was taken in EDTA coated vacutainer. Blood samples were drawn in heparinized syringes directly from heart of rats. On an Automated Hematology Analyzer (URIT – 2900Vet Plus, China), complete blood count was performed at hematology analyzer in NVL Islamabad. Each count presented the data of total white blood cells (WBCs) neutrophil, eosinophil, basophil, lymphocytes and monocytes. Erythrocyte count (TEC), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Hemoglobin concentration (Hb), Mean corpuscular Hemoglobin (MCH), and Hematocrit (HCT).

2.6 Histological analysis of brain

At the 7th day of treatment rats were anesthetized by (ip) administration of ketamix (0.1ml/kg body weight) and sacrificed to obtained body organs. The Brains were dissected out, weighed and subsequently immersed in freshly prepared 4% paraformaldehyde fixative for 16 hours at 4◦C and processed for paraffin embedding. The tissue blocks containing hippocampus (-3.4 to -3.8mm posterior to bregma) were further processed for paraffin embedding and 8µ thick serial section were cut in coronal plane under microtome. The section were stained with hematoxylin -eosin (H and E) conventional method and mounted. Hematoxylin- eosin was performed to investigate the structural changes following the buprofezin exposure and its protection with pre-treated atropine.

2.7 Behavioral analysis

To test motor coordination, fear conditioning, spatial learning and spatial navigation, learning and memory we used rotarod, horizontal bars, and parallel bars, passive avoidance test box, close maze or TSD maze and Morris water maze test respectively. Landing foot splay test was also performed to evaluate peripheral neuropathy. For nociception hot plat test and electric foot shock was also performed. Complete FOB (functional observational battery) testing were performed to assess whole behavioral profile of treated and control rats.

2.8 Motor coordination

Traditionally motor coordination has been evaluated in rats and mice by using rotarod test, which was performed by placing rats on rotating rod that rotates around its axis; the rat was allowed to walk forward to stay upright position and not fell off. We used

the set speed modified version of rotarods as both set speed and accelerating versions are commercially available. The other tests designated as horizontal bar, parallel bars and static rods these evaluate the coordination on static apparatus. The strength must be required for adequate performance on horizontal bars, especially of the forelimbs as the rat primarily grasps the bar with the front paws. We placed the rats about 25-30 min in experimental room before testing, to confirm they are well active. Allow the rats to return to their steel cages after performance of each motor test.

2.9 Rotarod Test

A rod of adjustable diameter (10.64cm) was rotated at about 4rpm. The rotation velocity was gradually increased until the rat fell off the rotating rod. When the increasing speed was measured, the time from start until the rat's fell off consider as measure of motor ability (Spyker and Avery, 1976; Rodier, 1978; Vorhees et al., 1979b). The motor coordination of control, buprofezin treated and pretreated atropine plus buprofezin rat was quantitatively evaluated using accelerating rota-rod for rats. With laser rpm calculator .The rotarod was 10.64 cm in diameter constituted of steel rod with knurled surface for treading. Two circular plastic glass disks were fixed at the ends of the rod with diameter of 40.64cm. The disks prohibit escape and worked as a barrier between rats. The rotarod was affixed 71.12cm above floor. Rats were mounted on the rotarod perpendicular to the long axis of the rod, with their heads facing away from the observer. As rat was mounted on the rod, the run experiment menu allowed the experimenter to run experiments. When the experiment was running the current speed of rota rod was displayed on LASER rpm calculator, as well as the amount of elapsed time as the experiment has started was measured with stop watch. As the rat falls from the rotarod, the rotational velocity of the rotarod at the time of the fall was showed by LASER rpm along with the amount of time the rat elapsed on rod was calculated manually by stop watch.

2.10 Horizontal bars test.

The "string test"1, "coat hanger test" or horizontal bar was used to measure forelimb strength and coordination. We have noticed that performance depends on tightness of string therefore most experiments use a metal bar. It was noted that rat's capability to grasp the bar was inversely proportionate to bar diameter and standardly used bar has 2 mm in diameter. The bar length was 91.4 cm and were lifted 73.6cm above the floor

by wooden supporting columns. We hold the rats by the tail, gently put it on the table in front of the apparatus, rapidly drags it backwards almost 20 cm so that rats aligns perpendicular to the bar, swiftly pick up and allowed it to grip the horizontal bar at its middle only with its forepaws, and fully relax the tail, consecutively starting the stop watch.

The criterion was to measure either a time of fall from the bar prior the rat reached one end of wooden column, or measurement of time until its one forepaw touched a supporting pillar.

The optimum test time was 30 sec.

2.11 Scoring procedure.

If rat fail to grip bar before first 5 sec this appear to be attributed to poor placing and this fall was not counted.

If rat fall between 1-5 sec score=1

If rat fall 6-10 sec score=2

If rat fall 11-20 sec score=3

If rat fall 21-30 sec score=4

If rat fall >30 sec score =5

2.12 Parallel bars

Two parallel steel bars 91.4cm in length and 4mm diameter were fixed 2.5 cm apart by wooden supporting columns at their ends elevated 73.6 cm above the floor. Two Parameters were measured. Maximum test time was 120 seconds. We hold the rats by the tail, gently put it on the bench in front of the apparatus, rapidly drags it backwards about 20 cm so that rats aligns perpendicular to the bar, swiftly pick up and allowed it to grip the horizontal bar at its middle only with its forepaws, and fully relax the tail, subsequently starting the stop watch.

1. First criteria point was to measure time the rat takes to orient 90◦ from start.

2. Second criteria point was to count the time taken by rat to reach at end of bar.

2.13 Fear conditioning

Fear conditioning tests were performed in fear conditioning test box comprised of light and dark compartments. Dark compartment contained electric bell for cued and steel wire floor connected with electric supply for contextual fear conditioning. Length of both chamber was 73cm each compartment was 36.8cm long and wide. Both chamber contained window of 10cm in diameter. Before experiment rats were permitted to acclimatize in testing room for 30 min.

For contextual fear conditioning test rats were placed in testing chamber and electric foot shock of 0.14mA was imposed for 2 seconds. Rats suddenly steps from shock compartment to other and freezing was examined for 5 minutes. For cued fear conditioning test rats were placed in same testing compartment and electric bell was tun on and electric current in steel wire was turn off, which delivered white noise tone of 90b dB for 30 sec. Rats suddenly stepped in from bell containing chamber into other compartment and freezing was examined for 5 min.

For both cued and contextual fear conditioning rats were retained in conditioning test box and allowed to acclimatize for 2 min. Every animal then received a white noise tone of 90dB for 30 sec and after that an electric foot shock of 0.14mA for 2 sec was subsequently delivered. The time duration between tone and shock was 2 min. After the exposure of last shock rats were left in chamber for 20 sec before removing. Freezing behavior was calculated for 5 min.

2.14 Evaluation of shock threshold for jumping, flinching and vocalization.

Shock threshold for flinching, jumping and vocalization was measured of control, buprofezin and pretreated atropine plus buprofezin in passive avoidance test box provided with variable current in mA.

2.15 Step through passive avoidance.

Rats were placed into passive avoidance test box containing a dark and light chamber. On the first day rats were placed into lighted chamber, and latency for the rat to step into dark chamber was recorded. Subsequently 0.2mA electric shock was provided for 2 sec and rat was removed. On second day rat were placed into lighted compartment, and latency for rat to steped into the dark chamber was measured again with maximum time of 5 min.

2.16 Spatial navigation, rearing and total locomotion activity measurements.

Spatial navigation rearing and total locomotion activities were measured by using TSD MAZE, CSS OR CLOSE MAZE.

2.16.1 Spatial navigation.

The activities of head direction cells (HD) hippocampal Grid cells, place cells, speed cells and TSD cells were assessed by preference of path selection by rats when food stimulus is baited in the both ends of path.

2.16.2 Rearing

Rearing behavior consist of rats standing upright position on its both hind paws .It is reflected an exploratory behavior. And it was index of anxiety in TSD OR CSS MAZE and the elevated plus maze. Yet there in no clear evidence that rearing is standard of either anxiolytic or anxiogenic behavior. Some studied suggested that increased in rearing was associated with increase anxiety level in rats. While others suggest that decreased rearing indicate increased anxiety. Rearing was used to differentiate anxiety like behavior from simplest ambulatory behavior.

In this study we have suggested that increase in rearing is associated with increase anxiety in Sprague dawley male rats. The maximum time for rearing was 15 min.

2.16.3 Total locomotion activity

The total locomotor activity of the testing animals is essential to distinguish prior to evaluation of TSD MAZE data or for any testing subject behavior maze. If locomotor capability was compromised due to treatment, then measuring activities that depend on on the capability of the animal to move was confused. The total locomotion activity of control, buprofezin treated and pretreated atropine plus buprofezin was analyzed manually after release of rats in 705cm long TSD maze. Rats were freely allowed to move in both long and short pathways. The total activity was measured in time scale of 15 minutes.

2.17 Use of TSD (time space determinant) maze, CSS or CLOSE maze to measure spatial navigation, working memory, References memory, locomotor activity and anxiety-like behavior in Rats.

Animal model have been proved valuable for researcher to give answer of question concerning the mechanism of behavior. The TSD maze is recently used platform to

measure in animal model especially in rats. It is relatively optimal test that provide a various information of behavior ranging from motor activity, emotionality and navigation cognition and memory of tested animal. As it concern to rodent model this method permits the study of different laboratory and wild strain of rats. This procedure will readily become useful to investigate the effect of different pharmacological compound on learning and memory as well as anxiolytic and anxiogenic effects.

2.18 Introduction

TSD (time space determinant) maze, CSS or CLOSE maze was recently developed by a student of physiology Muhammad Aslam Farkhi under the supervision of Dr. Irfan zia Qureshi in Qauid -I -Azam university Islamabad aimed to find the cells involved in earlier food seeking in rats given food stimulus in short and long pathway .He give these cell name TSD (time space determinant cells) and maze he used was given name as TSD Maze .CSS denote its shape. CLOSE refer to its close l1 and l2 pathways.

It has attained the status of most widely used maze for measuring psychological behavior of animal. It provided easy and fast analysis of well-defined behavior require a little training to test rodent and no special training for researcher conducting the test. These properties will lead to wide spread use of TSD Maze in research ranging laboratory rodent to wild rats. The reason from its superiority is that physiological and psychological concepts essential for these tests are straight forward and well comprehensible.

A TSD maze comprises of a wall-enclosed area that is of adequate height to prevent the escaping of rats. The shape of maze was long and short zig zag in CSS shape with enough area, depending on the size of the testing animal. Several parameters can be recorded in the TSD Maze with maximum parameters including various kinds of locomotor activities. Most commonly Ambulation behavior is studied but others such as rearing or latency can also be recorded.

Mostly bare maze is used in analysis of rodent behavior. Thus, the addition of rats, either of given pathway, adds the capability to see how the rats navigate in food stimulus in in less time and space arena. Significant parameters when rodents are accessible are typically thigmotaxis, fecal boli, rearing, emotionality and anxiety like behavior.

Many tests of anxiety related behavior are established on the body activity of testing animal and locomotion. However interpretation of emotionality behavior from non-emotional, such as motor activity, has been matter of investigation. As the TSD maze was initially designed, two parameters of emotionality was assumed, fecal boli deposits or defecation and locomotor activity. However, studies have shown that these two measures provide unrelated supporting conclusion as emotionality was multidimensional in rodents. However, there are differences in the research about these parameters emotionality or anxiety in rodent models. Investigator conclusively relate results from analysis of TSD Maze with other procedure of anxiety while comparing rodent models.

Fig TSD maze, CSS maze or Close maze

2.19 Preparation of Room for Testing and Close maze Apparatus.

1. Use a two unit TSD Maze comprising of two zig zag short and long pathways was used for this analysis. Long zig zag pathway measured 391.1cm (length) x 10cm (width) x15 cm (height) and was made from high density glass and short pathway was measure 313.9 cm. Total length of both pathways was 705 cm.

2. TSD Maze was consisting of a door of 5cm in diameter with additional windows in roof of pathways at the end of both pathways.

3. Maze floors was Texture for traction throughout ambulation however maze walls were kept smooth. Maze pathways were fully empty for the performing the test. In concern of the rest of this protocol, both pathways of the maze mention above will be used to represent the TSD Maze.

4. Prior to use wipe the both pathways before tests with a 95% Ethanol and eradicate any scent secreted by the previous testing rat.

5. Wait some time before testing rat to evaporate ethanol completely. This may take5-10 min between each testing session.

6. The analysis was manually performed due to unavailability of video tracking camera and software.

7. As experimenter, be sure that there should be enough space in the room to be entirely imperceptible by the rodent being tested in the maze so that rat's behavior may not influenced.

2.20 Administration of the TSD Maze Test

1. Bring the rat from housing room into their testing room in steel cages. Prior to starting the test. Allow them to acclimate to the procedure room for at least 30 min. **2**. Gently grasping rat by its tail remove it from the cage and place the rat in the front door of TSD maze while simultaneously observing the behavior of rats and carry stop watch for time measurement. Normally the rats move instantly to the boundary walls of the maze and the timing of release and food navigation of the rat should recorded to measure this movement.

3. Allow the testing rat to move freely and continuous throughout the respective pathway of the maze for a 15 min period during this time, the observer track the distance and time.

4. Pick up the rat gently at end of the test period, remove it from the maze and return it to its steel cage.

5. Before cleaning the Maze manually count the fecal boli found in Maze. **6**. Wipe up all spots of urination after removing all fecal pellets and Spray the floor and

walls of the maze pathway with 95% ethanol. The ethanol solution should be completely dried before testing next rat.

7. Repeat this method for all rats.

The TSD Maze is one of the most recently used protocol for studying behavior of animals. During the TSD maze performance multiple important conventional and ethological measures are composed and evaluated. These data obtained from TSD maze allowed the investigator to analyze behaviors such as locomotor activity to rearing, anxiety, thigmotaxis, object recognition, spatial navigation, learning and memory. However there are some shortcoming in use of TSD maze. One confusing matter is the multiple static variables that must be manipulated while performing tests. For example time, novel object inclusion and lighting conditions. In spite of these problems, the TSD maze considered as one of the most efficient technique in rat's behavior research.

Here, four features of TSD or CLOSE Maze are readily characterized while behavioral study using this procedure.

1) During entire testing session the measurement of distance (cm) covered by rats.

2) Thigmotaxis or wall hugging behavior it is measure of anxiety and characterize by the time rats remain adjacent to wall of TSD maze for time duration of 15 minutes.

3) Counting the number of fecal pellets in each pathway after the removal of rats.

Defecation is a negatively associated with emotionality in rodents and can be used to specify levels of anxiety in the rats.

4. Spatial navigation depend upon path preference by rat when food stimulus is baited in ends of both l1 and l2 short and long pathway. Short pathway preference is indication of normal function of hippocampal grid cells, place cells, head direction cells and specially TSD cells. Working memory and reference memory.

It should be noticeable that some investigators have inferred high activity or increase exploratory behavior is a measure of low emotionality however others perceive that exploratory behavior doesn't depends on emotionality. We have conceived during our experiment that exploratory behavior doesn't depends on emotionality.

One has to appreciate that differences in locomotor activity can distinguish measurement of emotionality. So far, as total ambulatory distance was related in same rat strain, however mice activity level were distinct from emotionality factors.

Rearing behavior can define as standing of testing animal in a vertical upright position on both hind paws. It is measured an exploratory behavior and used as index of anxiety in both the Elevated Plus Maze and TSD maze. Some studies specify increased rearing is in associated with increased anxiety levels in rat while others suggest decreased in rearing is indication of increased anxiety. Thus, rearing can distinguish anxiety-linked behaviors from other ambulatory behavior. It has been suggested that anxiety analysis in rats was much more complex than using a single parameter in only one maze. Thus, using numerous trials or several parameters in a single test can support the results assessment.

Thigmotaxis behavior is perceived largely in rodents and is associated to anxiety-like behaviors. Irrespective of the principal cause, thigmotaxis is an essential anxiety linked behavior and often recognized as the initial point for further precise anxiety tests. In TSD maze Thigmotaxis was used to measure anxiolytic, anxiogenic and even non-pharmacological actions. Anxiety linked drugs such as chlordiazepoxide and diazepam have revealed substantial effects on rats behavior in the TSD however dopamine agonists have revealed that dopamine receptors like D1 and D2 cause anxiogenic-like effects due to high dopaminergic transmissions..

In the TSD Maze there is significant strain differences in response to rat anxiety-like behavior. It was also clearly reported that highly emotional rats showed more defecation. Recently it has been reported that defecations may definitely be a valuable index of emotional anxiety-related behaviors in short testing periods as accomplished here as compared to long testing (30 min) where minor variation in responses are found.

In the TSD Maze the behavior of rats depends on their tactile sensations. Therefore any damage to or shortage of whiskers of rats may results a substantial decrease in anxiety-linked behavior measure as the rat fail to contact with maze walls.

We debate the use of the TSD Maze as it was associated with motor locomotion of the rat being tested, other behaviors tests can also be performed in TSD such as memory and novel object recognition. Time in the TSD maze with a novel object recognition can range from 15 min to 30 min and depends on type of memory being analyzed. Due

to flexibility and simplicity of the TSD maze in the novel object recognition permits for short- or long-term memory testing, and can be used to evaluate the effect of acute drug administration on a particular stage of memory formation. Briefly, the TSD Maze test was an optimum measure of performance.

2.21 Evaluation of working and reference memory and errors in TSD or CSS Maze

Working memory can be demarcated as a memory for an object, recognition, or location that was used within a testing period, but not usually between the periods. It is variant from reference memory which was demarcated a memory that would typically be attained with rehearsal training and would sustain from days to months. The reference memory was mostly the memory for the 'rules' of a given chore. For instance, when testing object press a bar receive a food object or a water maze established with a hidden platform or entrances into the food containing pathway of the TSD CSS Maze. Moreover, working memory enable the testing object to remember which pathway it had visited in a testing period.

2.22 Testing animal adaptation period

The rats were presented two periods of adaptation on two succeeding days before the learning process commences. The testing rats were allowed to walk around the food baited pathway of the maze for 15 min during the testing time. The testing rats were explored the TSD Maze baited with food stimulus first in long pathway, then food baited in short pathway and at the end food baited at both pathways and path acquisition in each case was recorded. Following the adaptation period, the acquisition process was ongoing.

2.23 Testing animal Acquisition career.

During the testing animal acquisition career or (learning session), the rats were assumed three trials of acquisition per day until the rats achieved the learning criteria. The learning criteria were confronted as follows. The trial was sustained for 15 min and the training was continuous until the rats achieved the criteria of 80% correct choice; i.e., at least four correct entries out of five. The duration of this session varies depending upon condition of research procedure the maze was washed with ethanol (70%) at start of trial session and thereafter one path was baited with food stimulus. For first trial the rat was kept in central box and was permitted to choose any pathway. When a rat reached the end of pathway and ate the bait reward, the path choice was noted. Only

the first approach to the baited pathway was documented as a correct choice and the maze pathway. For second trial the pathway was rebaited and entries of rat in baited pathway was recorded. Entrances into the path containing no food stimulus were recorded as Reference Memory Errors (RME).For third trial the both pathways were baited with food stimulus and path entries of rat was recorded reentries into baited pathway when both pathways are baited referred as WME. For fourth trial the both pathways were baited and choice of short pathway was recorded as correctness of TSN. Each rat was assumed four trials per day and obtained data from the four trials were averaged and included in analysis of final data. The performance pattern of rats were recorded by the percentage of the correct choices, RME and TSN (time space navigation) in TSD Maze, COSE Maze or CSS Maze.

2.24 Morris water Maze Test.

A neuroscientist Richard G. Morris initially designed the Morris water maze in 1981 for testing hippocampal-dependent memory containing long-term spatial memory and acquisition of spatial memory.

2.25 Preparation.

A circular steel pool of diameter 125cm blue color was used for testing. The pool was divided into four quadrant each with circumference of 208cm.The height of pool was 60cm and water level in visible plate form was 10cm.we place the platform of 12cm in diameter and 40.6 cm in height in pool. We fill the pool in water until the water level was 1cm below the platform. Allow the water to equilibrate at room temperature 22°C. Add the hot water to maintain temperature

2.26 Testing method.

1. We removed the rat from their cages to behavior room. Rats were kept in area where they were not allowed to see the pool or spatial cues.

2. Before testing allowed them to acclimatized for at least 30 min.

3. To increase visibility we place the flag on platform.

4. For testing we lift the rat from their cage by the tail and softly put the rat into the water opposite to the edge of pool.

5. Allow the rat to remain on the platform for 5-6 sec if the rat find the platform before 60sec then transfer it to home cage.

Repeat the procedure for each trial.

For visible platform (nonspatial) test. Rats were given four trial. For each trial the order of start position was changed. The platform search latency for each rat was recorded with stopwatch. The maximum time for rat to find platform was 60 sec.

For hidden platform (spatial) water level was raised 1cm above the platform and rat were trained to locate hidden platform. It was used for long term memory and acquiring spatial location of platform. Rats were given four trials and latency for rat to locate platform was measured in duration of 60 sec.

For probe trial we create one trial and plate form was removed. There was one starting direction farthest from platform quadrant. Platform search time and no of platform crossing was recorded for 60 sec. The rat starting direction and platform location in Morris water Maze was as follows.

	Day 1		Day 2 probe test
	Platform location	Starting direction	NO platform. Start direction
Trial 1	SW	S	N
Trial 2	NE	N	
Trial 3	NW	E	
Trial 4	SE	W	
Trial 5	CENTER	E	

TABLE 1 WATER MAZE PROTOCOL USED.

2.27 Hind limb landing foot splay test

Landing foot splay was evaluated according to the procedure described by Bowen and co-worker. The tarsal joint of each hind foot of each rat was marked with a drop of colored ink and animal was held in a supine position and then dropped from a height of approximately 30 cm on to a white absorbent paper. The distance (cm) between two

foot prints of hind limbs was measured. This procedure was repeated three times for each animal and finally three readings were averaged.

2.28 Physiological evaluations

2.28.1 Body weight

Body weights of individual male rat of control group and treated were recorded at 7th day of experiment and averaged.

2.29 Hot Plate Tests

It is a simple behavioral test design by Eddy and Leimbach (1953) used for detection of the effects of chemical on the threshold for perceiving pain.

2.30 Procedure

First we switch on the hot plate until the temperature of plate reached at 60◦C.we put the cylinder shaped jar on hot plat.

Cylinder was cleaned with disinfectant (70% alcohol). Stay for 2 min to re-establish new temperature before onset of testing.

We put the first rat on plat and rapidly start the stopwatch to calculate the hind paw withdrawal latency.

We stop the stopwatch after the rat express any reaction to heat (paw licking shaking jumping or any other reaction.

Latency by default was consider when does not show any reaction to heat after 30 sec.

Keenly observe any type of aversion behavior licking, shaking and jumping of hind paw and measure the latency of control and treated group.

After accomplishing the test rats were placed into their room and apparatus was thoroughly cleaned.

2.31 Functional observational battery (FOB)

Functional observational battery (FOB) was conducted to measure the behavioral, autonomic, physiological and neurological functions of control, buprofezin treated and pretreated atropine plus buprofezin. The FOB evaluations in this study were based on

a standardized method validated and followed by the laboratory. The FOB comprised of six types of observations.

1. Home cage observations.

2. Hand held observations.

3. CLOSE maze measurements.

4. Sensory functions.

5. Physiological evaluations and

6. Neuromuscular measurements.

1. Home cage observation include following parameters.

Posture

Vocalization

Respiration

Activity level

Convulsions

2. Hand held observation include following parameters.

Lacrimation

Salivation

Piloerection

Palpebral closure

Limb grasping

3. CLOSE Maze measurements include following parameters.

Gait

Ambulation

Rearing

Arousal

Fecal boli count

Involuntary motor movement.

Time space navigation

4. Sensory function include following parameters.

Touch response

Palpebral reflex

Tail pinch response

Pupil light response

5. Physiological evaluation include following parameters.

Body temperature

Body weight

6. Neuromuscular measurement include following parameters.

Abdominal tone

Limb tone

Flexor reflex

Extensor reflex

Forelimb hopping

Landing foot splay

Righting reflex

Statistical analysis.

The data was statistically analyzed with ONE WAY ANOVA followed by Tukey's post hoc multiple comparison test. Data was demonstrated as Mean ±SEM. $P<0.05$ represented the significant difference.

RESULTS

3.1 Buprofezin induced Histopathological alterations of hippocampus were attenuated by pre-treated atropine

MWM test and TSD maze finding demonstrated that acute Beprofezin exposure impair spatial, working and reference memory as well as time space navigation. We concentrated on histopathological alteration of hippocampus provoked by buprofezin treatment. Hippocampus can be classified into several regions on the basis of pyramidal neuron morphology as CA1-CA4.We observe the changes in hippocampus with staining. Results demonstrate that buprofezin exposure induce alteration in various regions of hippocampus. It is reported that the function of pyramidal cells in CA1 region were attributed with long term memory and degeneration of CA1 pyramidal cells may cause significant memory loss. Thus to analyze histopathological alteration and ultrastructure variation in response to buprofezin exposure CA1 and CA3 regions of hippocampus was analyzed by hematoxylin and eosin staining.

H and E staining showed that in control group pyramidal cells were arranged orderly with intact nuclei stained clear, dark blue. However in acute buprofezin treated rat's hippocampus H and E staining revealed more degenerative (apoptotic) neuron and pyramidal cells showed granular vacular changes and nuclear pyknosis. The cell junction and basement membrane was degenerated. In pre-treated atropine group pyramidal cell show better cell morphology compared to buprofezin treated. Cells junction were intact and cells were in compact form. Only few cells underwent degenerative changes. These finding clearly demonstrate the ameliorative effect of pre-treated atropine against buprofezin neurotoxicity.

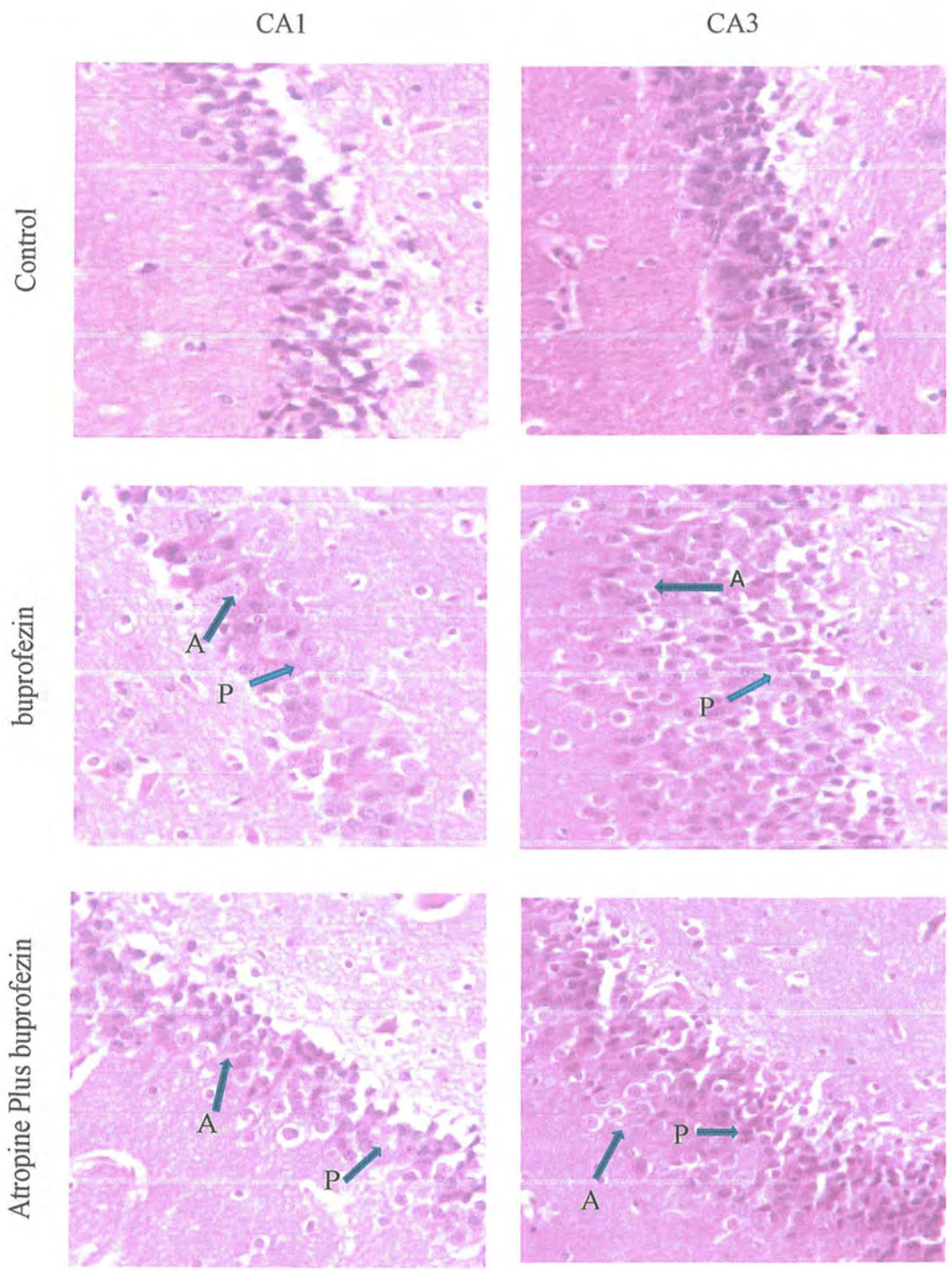


Fig 3.1. Photomicrograph of 8μm thick coronal section of rat hippocampus (-3.8 mm behind bregma) stained with hematoxylin-eosin (HE).The panel of each column showed control, buprofezin treated and pre-treatred atropine plus buprofezin of CA1 and CA3 region of hippocampus.(A) showed the apoptotic pyramidal cells.(P) showed the pyknotic cells. Normal cells are darkly stained. Disrupted and misalignment of layers is shown in experimental group. However pre-treated atropine group showed less pyramidal neuron degeneration compared to buprofezin treated group.

3.2 Atropine reverse the impaired motor coordination induced by acute buprofezin exposure.

Motor coordination of rats was analyzed by using rotarod. The results indicated that latency of fall from rotating rotarod was significantly reduced ($p<0.001$) in acute buprofezin exposed groups compared to control and pre-treated atropine counteract the toxic effect of buprofezin as there is less significant difference compared to control. As shown in Fig 3.2.

3.3 Forelimb grip strength

The forelimb grip strength and coordination were assessed by using horizontal and parallel bars. Results have shown statistically significant ($p<0.001$) impairment in fore limb grip strength in horizontal bars and was no significantly counteracted by atropine. Fig 3.3,4,5,6.

In case of parallel bar time taken by rat to orient 90◦ and traverse time was significantly increased in buprofezin treated rats compared to control. Total distance on bars in given time duration was reduced in buprofezin exposed group. Pre-treated atropine reversed the buprofezin effect as the there is no significant difference between control and pre-treated atropine plus buprofezin groups.

3.4 Buprofezin induced long term deficit of contextual and cued memory is revered by Atropine.

Groups of rats were trained for behavior testing. Two sets of stimulus a mild foot shocks with an associated auditory cues were administered to rats. After training session, we found that buprofezin treated rats exhibit significantly less freezing compared to control and pre-treated atropine plus buprofezin exhibit no significant change in freezing compared to control. These results suggested that pre-treated atropine counteract the loss of fear conditioning caused by acute exposure of buprofezin. Initially we tested the cued then context and the lastly, we paired cued and context to examine the fear conditioning. Fig 3.7,8,9.

3.5 Atropine slightly counteract the impaired passive avoidance induced by acute exposure of buprofezin.

In passive avoidance test the rat learned to decrease their natural tendency to avoid from light compartment and step into darker compartment of training chamber. We trained the rats by placing them into a lighted compartment and measured the latency after which the rat entered into dark chamber where they receive a mild electric foot shock. Next day we assessed the latency of rat to step into dark compartment. Our results suggested that on first day rat spend significant greater time in lighted compartment compared to control. While on second day in spite of receiving foot shock buprofezin treated rat latency in lighted compartment was significantly reduced compared to control and was remarkably reversed by pre- atropine administration. These finding indicate the loss of sensory receptor by acute buprofezin exposure. Fig 3.10.

3.6 Quantification of shock threshold for flinching, jumping and vocalization.

Our results have shown the remarkable increase in shock threshold for flinching, jumping and vocalization in buprofezin treated rats compared to control indicating loss of general sensory perception. Pre- treated atropine rats showed no significant increase in shock threshold compared to control. Fig 3.11.

3.7 Effect of buprofezin on spontaneous behavior and impact of atropine.

Spontaneous behavior testing were performed in TSD maze for 15 min on adult male rats. Total locomotion activity and rearing mean was measured in all testing rats. Results showed the rats who received acute exposure of buprofezin exhibit a significant decrease in locomotion and rearing activity and pre-treated atropine rats represent no significant difference compared to control reveals reversal of buprofezin toxicity by atropine. Decrease number of rearing represent the anxiety and depression behavior in buprofezin treated rats. Fig 3.12,13.

3.8 Buprofezin induced impairment in working memory, reference memory and spatial navigation is counteracted by atropine

3.8.1 Correct choice of path during acquisition career.

Results were obtained by using ONE WAY ANOVA followed by Tukey post hoc multiple comparison test. Our finding have reveals a significant loss of correct path choice during acquisition session in buprofezin exposed rats compared to control. Rats were subjected to five trial for each and percentage of correct choice was calculated. At first day the control group was unable to reach 80% correct choice of path. Second day

after continues trial rats ultimately obtained 80% correct choice of path. Impairment of correct path choice was counteracted by pre-treated atropine. Fig 3.14.

3.8.2 Correct choice of path during navigation session.

In time space navigation session the rats were trained to obtained food stimulus from shorter pathway to reach maximum 10% of correct choice although food is baited in both pathways. We have found less than 10% of correct choice of control rats on first day and buprofezin treated rats showed significantly less correctness compared to control. Moreover pre-treated atropine has reversed the effect. On second day control rats achieved the criteria of 10% correctness after five trials of training. Buprofezin again decreased the choice correctness. These finding suggested the deterioration and apoptosis of TSD, grid cells, speed cells and hippocampal place cells following the acute exposure of buprofezin. Fig 3.15.

3.8.3 Working memory and reference memory error during acquisition are attenuated by pre-treated atropine.

3.8.4 Working memory error

Working memory is memory of object stimulus or recognition of location used in testing session. Therefore if rat enter in food baited path it is working memory correctness and if it reenters into baited pathway when both pathways are baited it is referred as working memory error. On first day of training the working memory errors (WME) were greater in buprofezin treated rats compared to second day. Results suggested that continuous training alleviate the incidence of working memory error and pre-treated atropine significantly attenuate the working memory errors. Fig 3.16.

3.8.5 Reference memory error

Reference memory is memory for rule of given condition. For example acquisition of baited path provide food to rats. Entries of rat into pathway with no food stimulus is referred as reference memory error .Therefore results suggested that reference memory error (RME) during acquisition session changed days after training. Buprofezin treated group exhibited more reference memory error compared to control group. On second day (RME) were comparatively greater reason is still not known. However there was no substantial difference in control and pre-treated atropine group were found. This

demonstrate the reversal effect of pre-treated atropine on reference memory errors. Fig 3.17.

3.9 Atropine attenuate the deficit in spatial learning induced by acute exposure of buprofezin.

We evaluate the spatial learning of testing rats of all groups using Morris water maze test. In our training procedure animal undergoes four trial of a day. Using spatial cues in room rat were allowed to find visible and hidden platform located in one quadrant of maze. In training process latencies to find platform continuously decreases in treated and control groups. The latency of rats in one quadrant of pool was measured. Results have shown that buprofezin treated rats spend significantly more time in one quadrant while searching platform in all trial represent deficit of spatial learning. However no significant difference was found between control and pre-treated atropine plus buprofezin. Fig 3.18,19.

While probe testing the platform was absent the time spent in searching target quadrant was significantly less in buprofezin treated rats compared to control. However atropine weakly revers this effect as there was substantial difference between control and pre-treated atropine plus buprofezin. Fig 3.20.

Similarly the number of platform crossing were remarkably less in buprofezin exposed group compared to control. Pre- treated atropine strongly counteract and number of crossing were increased near to control as there is no significant difference between control and pre-treated atropine plus buprofezin group. Fig 3.21. Thus buprofezin treated rat devoid the spatially selected search strategy and were unable to found the specific location of hidden platform and effect was counteracted by pre-treated atropine. Together these results demonstrate that buprofezin induce various form of hippocampal dependent memory defects as analyzed by fear conditioning, passive avoidance and the Morris water maze.

3.10 Hind limb landing foot splay test.

Hind limb landing foot splay analysis of all groups was performed on 7^{th} day. Results revealed a significant decrease in foot splay in buprofezin treated rats compared to control and no significant difference was found in pre-treated atropine and control rats.

The decreased foot splay may be due to skeletal muscle weakness because of peripheral neuropathy. Fig 3.22.

3.11 Hot plat test to evaluate analgesic effect of buprofezin and effect of pre-treated atropine.

Hind paw lick latencies were investigated of all groups. Results showed that rats exposed to acute buprofezin exhibited significant increased latencies to hind paw lick compared to control and there was no difference in pre-treated atropine and control. buprofezin treated rats response to nociception of heat were lost and was contracted by pre-treated atropine. Fig 3.23.

3.12 Body weight evaluation

Body weight of buprofezin exposed group was comparable to concurrent control and pre-treated atropine group at 7^{th} day of treatment. Treatment related a significant reduction in body weight. Pre-exposed atropine slightly attenuate the weight loss induced by buprofezin. Fig 3.24.

3.14 Mechanism of buprofezin Neurotoxicity

Buprofezin intoxicated rats died soon after the administration of neostigmine (30µl/kg/day i.p) a blocker of AChE. It suggested that a small concentration of AChE in synapse was dominantly occupied by a small concentration of neostigmine consequently tremendously elevated the ACh level in synapse leading to tremor and death of rat. However pre-treated atropine delayed convulsion, tremor and death indicated a protective role of pre-treated atropine against buprofezin induced neurotoxicity. Pre-treated neostigmine plus atropine also delayed death due to sufficient availability of AChE in synapse that metabolizes the Ach. Pre-treated atropine plus neostigmine treatments in non buprofezin intoxicated rats also cause sudden death due to plenty of ACh in synapse as AChE and cholinergic receptors have been blocked.so these results suggested that buprofezin increases the concentration of ACh in synapse by inhibiting the synthesis and release of AChE due insufficient supply of ATP. Fig 3.25

3.13 Hematological evaluation

Results are demonstrated in table 3.1. We compared the all groups on 7^{th} day and observed that buprofezin treated group showed significant increase in white blood cell count (WBCs) ,lymphocytes, monocytes, red blood cells distribution width (RDW) and

platelets. Pre-treated atropine group increased in WBCs count and decreased in lymphocytes, monocytes and (RDW).platelets were increased in pre-treated atropine group. **Table 3.1.**

3.14 Functional observational battery (FOB)

Functional observational battery was performed to analyze behavioral, autonomic, physiological and neurological function of all tested rats at 7th of experiment. In this study the (FOB) analysis was based on standardized method issued by laboratory. For example for righting and landing foot splay rats were allowed to fall on well cushioned surface. The (FOB) comprised of six types of observations. **Table 3.2.**

1. Home cage observations
2. Hand held observations
3. Close maze observations
4. Sensory observations
5. Physiological and neuromuscular observations.

3.14.1 Home cage observations

Rats of control group exhibit a normal, posture, activity, vocalization, respiration and convulsions. Whereas rats treated with acute exposure of buprofezin demonstrate abnormal body posture and activity level compared to control. Atropine have slightly attenuate the buprofezin effect. The abnormal posture and activity of rats investigated in this study might be associated with impaired somatosensory processing damage to neuromuscular junction following acute exposure of buprofezin.

3.14.2 Hand held observations

Buprofezin treated rats showed reduced reactivity to handling and pre-atropine treated rats exhibit remarkable reactivity to handling. Other autonomic functions such as lacrimation, salivation, piloerection and abdominal tone were decreased in buprofezin treated rats compared to control. However pre-treated atropine reduce the intensity.

3.14.3 Close maze (TSD) observations

Rats of all groups were assessed for their performance in close maze by using their various neurobehavioral, locomotor, neurological and sensory-autonomic

measurements. Buprofezin treated rats' exhibit significant altered ambulation, gait, rearing, arousal, fecal boli count and time space navigation. However pre-treated atropine have shown to ameliorate these neurotoxic effects.

3.14.4 Sensory observations

Buprofezin treated group showed slow response to touch, tail pinch, palpebral and pupil light response. Buprofezin produce distal sensory motor neuropathy of the central and peripheral nervous system this may result alter sensory motor function of rats and is reversed by pre-treated atropine.

3.14.5 Neuromuscular observations

Rats of all groups were analyzes for their neuromuscular performance. Buprofezin treated group exhibit decrease in limb tone, flexor reflex, extensor reflex, landing foot splay and righting reflex. Pre- treated atropine attenuate these neuromuscular alterations.

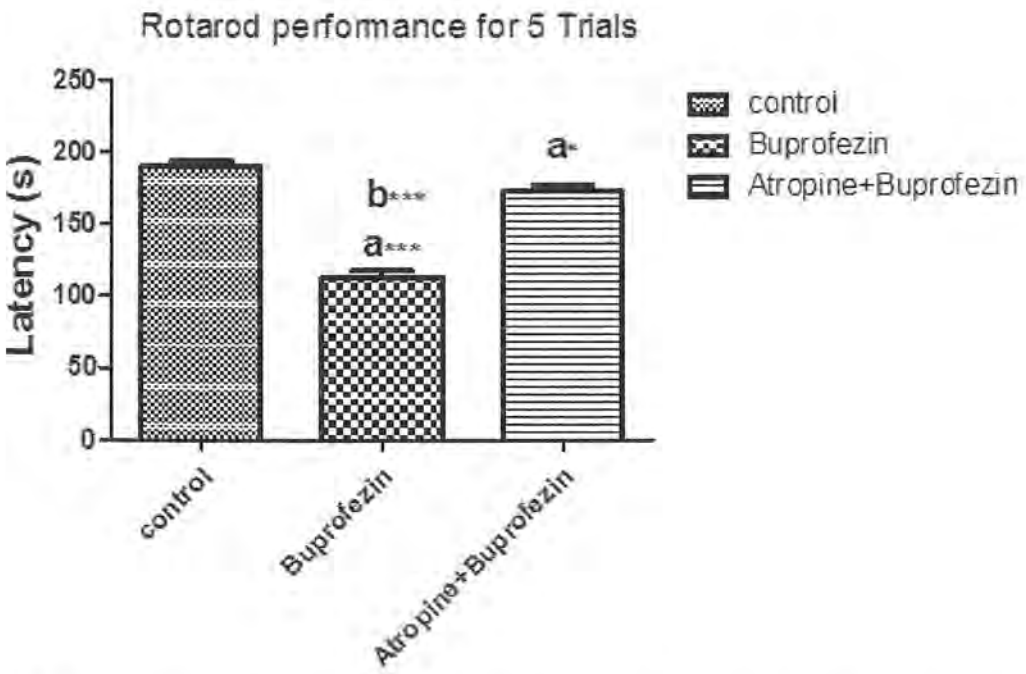


Fig 3.2. Plot of latency time spent on rotating rotarod for control, buprofezin treated and pre-treated atropine plus buprofezin in adult Sprague-dawley male rats. Note the decrease in latency of buprofezin treated comparison to control and pre-treated atropine counteract the effect. However there is still substantial difference between control and pre-exposed atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, ***P<0.001

a, significant different from control

b, significant different from pre-treated atropine.

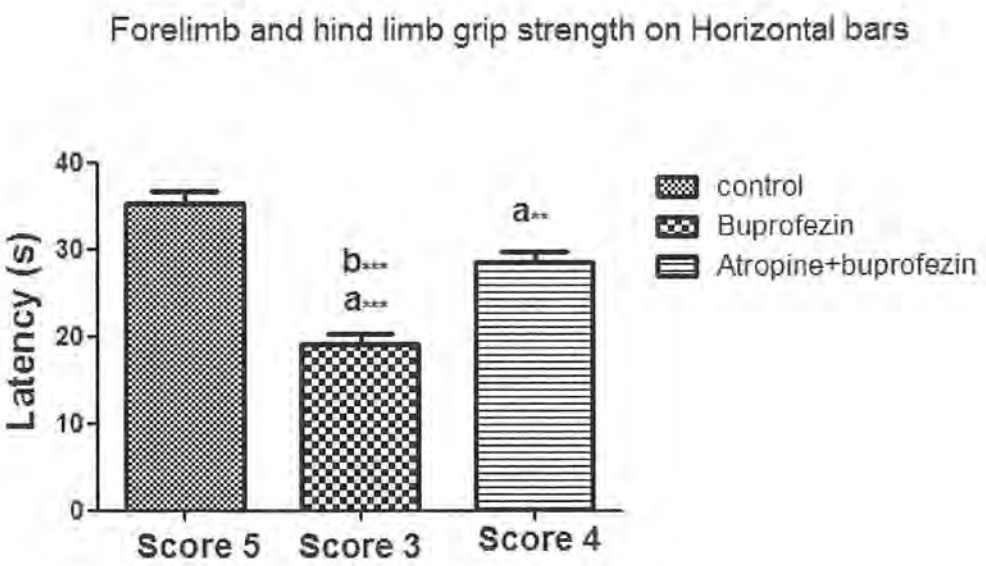


Fig 3.3.plot of forelimb and hind limb grip strength on horizontal bars or all testing groups of adult Sprague-dawley male rats. Note the significant decrease in score of rats treated with buprofezin compared to control and pre-treated atropine slightly counteract the effect. There is still substantial difference between control and pre-exposed atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, **p<0.01, ***P<0.001

a, significant different from control

b, significant different from pre-treated atropine.

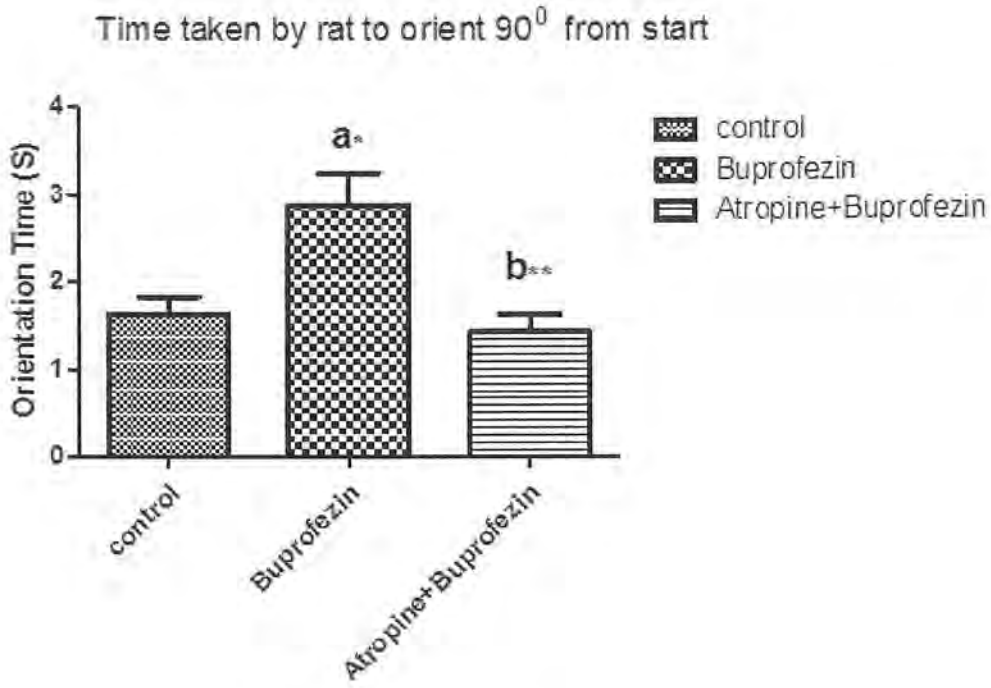


Fig 3.4. The plot showed time taken by rat to orient 90° from start in parallel bars performance. Note the buprofezin treated rats take significant more time for orientation compared to control. Pre-treated atropine completely reverse the effect as there is no difference between control and pre-treated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01

a significant different from control

b significant different from pre-treated atropine.

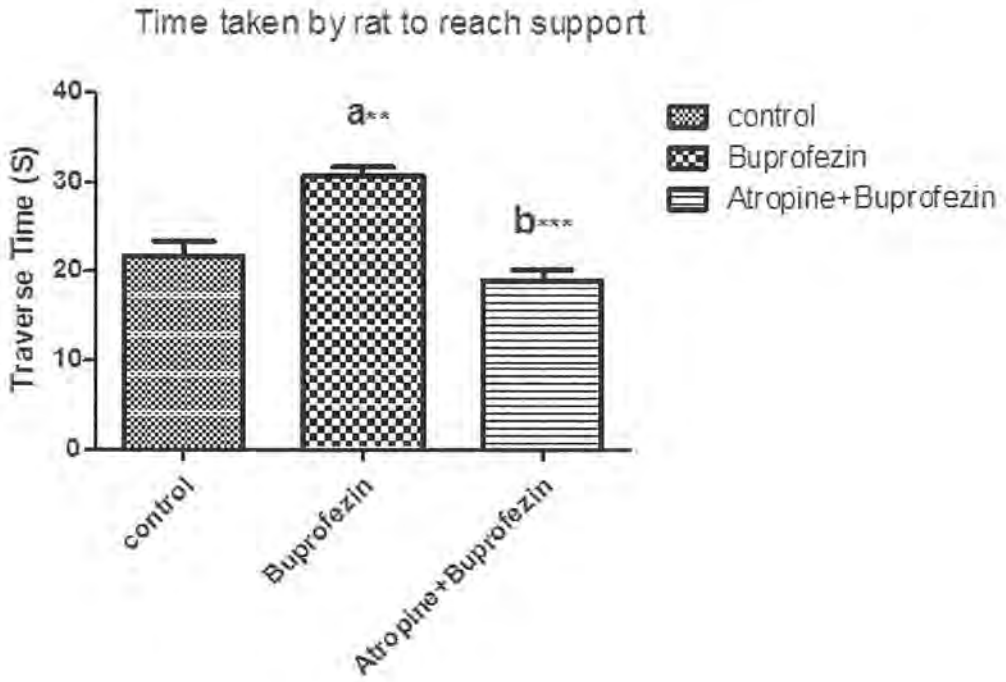


Fig 3.5. Plot represent the effect of buprofezin, and pre-treated atropine on traverse time (time taken by rats to reach support on parallel bars).Note the buprofezin treated rats take significant more time to reach support while pre-treated atropine rats exhibit no difference compared to control.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01

a, significant different from control

b, significant different from pre-treated atropine.

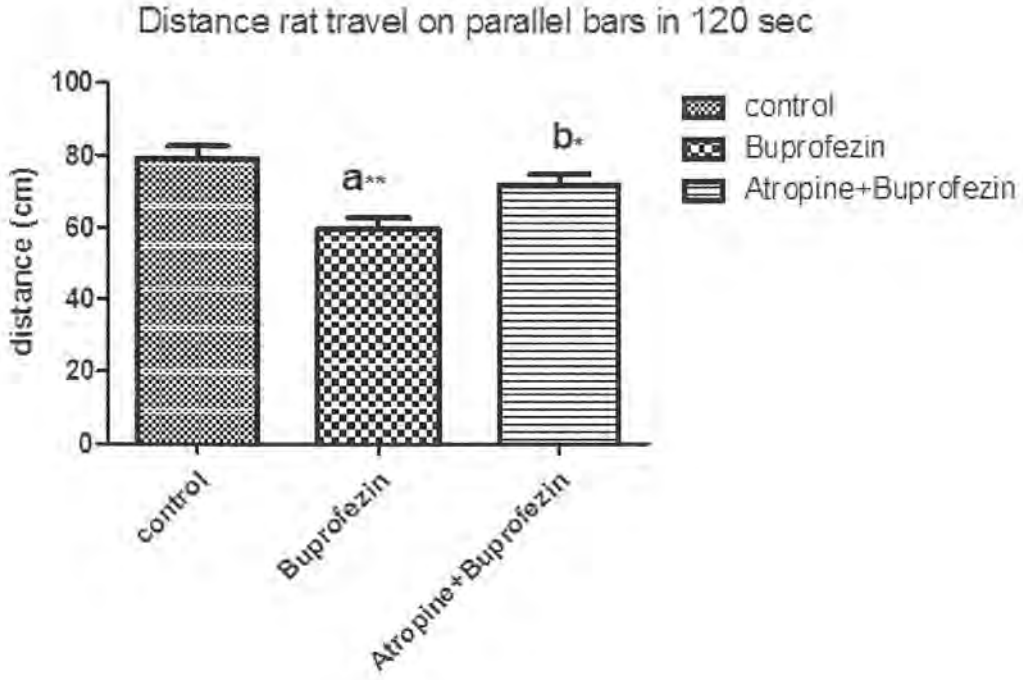


Fig 3.6.plot demonstrate the effect of buprofezin and pre-treated atropine on the total distance traveled by rats on parallel bars in 120 seconds. Note the rats treated with acute exposure of buprofezin cover significantly small distance in given time duration compared to control. Pre- treated atropine attenuate the effect and exhibit no difference with control.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01

a significant different from control

b significant different from pre-treated atropine.

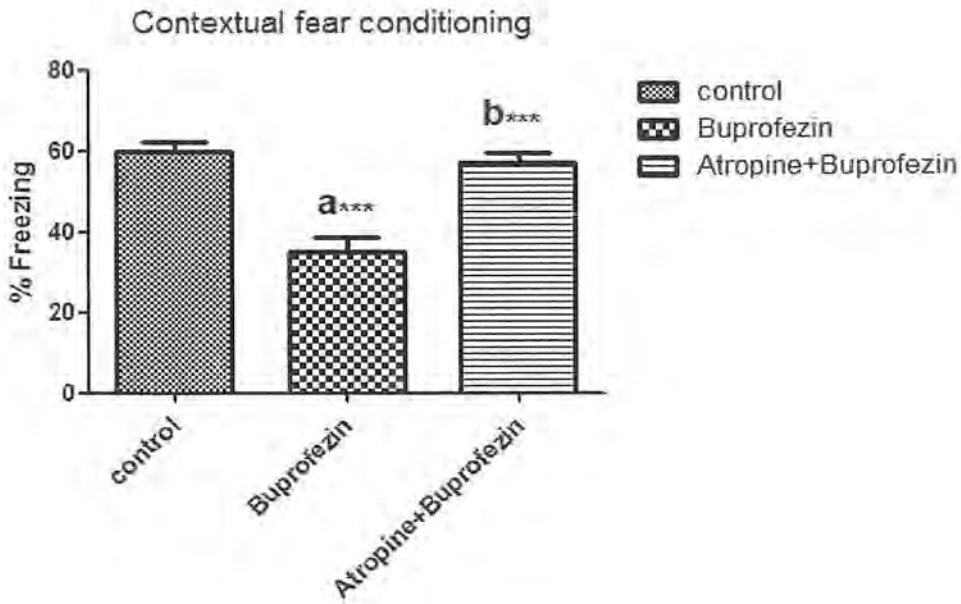


Fig 3.7.plot showed the effect of buprofezin and pre-treated atropine on contextual fear conditioning behavior of rats. Note the buprofezin treated rats have fear contextual fear conditioning deficit and pre-treated atropine reverse the fear conditioning as there is no difference in control and pretreated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, ***p<0.001

a significant different from control

b significant different from pre-treated atropine.

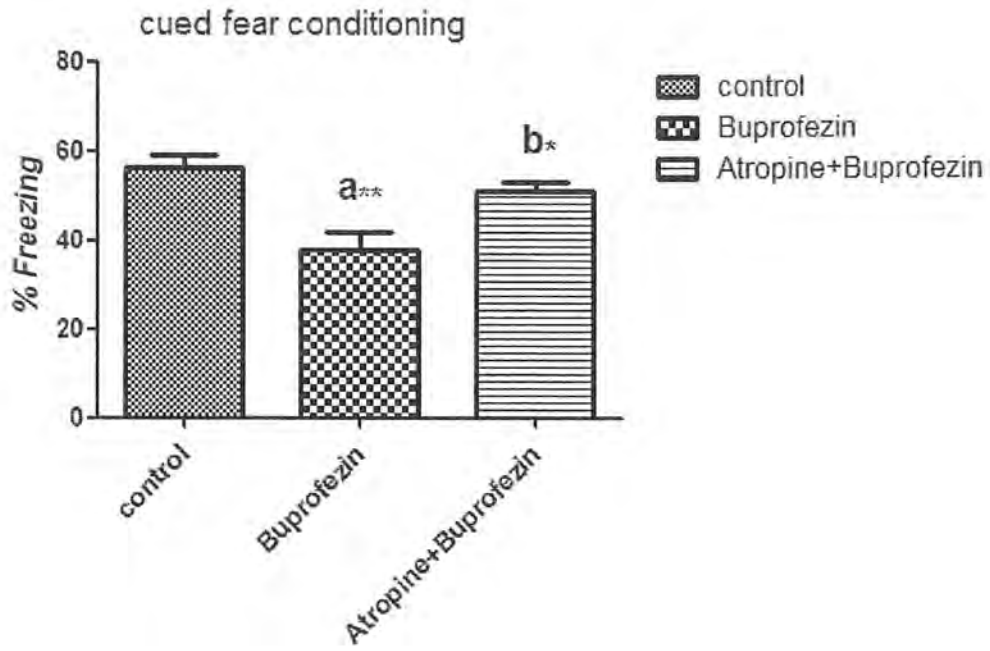


Fig 3.8.plot showed the effect of buprofezin and pre-treated atropine on cued fear conditioning of rats. Note the buprofezin treated rats exhibit cued fear conditioning deficit compared to control and pre-treated atropine has remarkably reverse its effect.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, **p<0.01

a significant different from control

b significant different from pre-treated atropine.

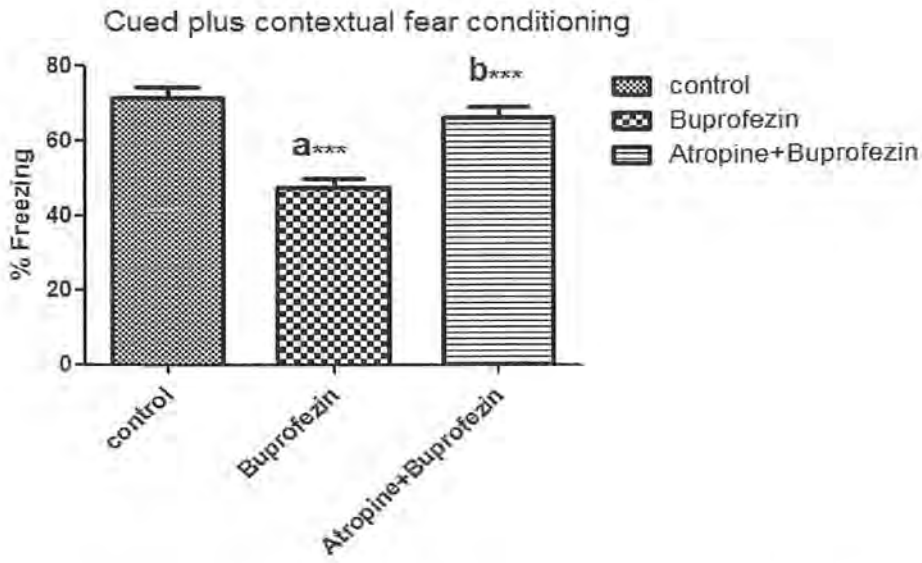


Fig 3.9.plot exhibit the acute effect of buprofezin and pre-treated atropine on cued plus contextual fear conditioning. Note the buprofezin treated rats showed both cued and contextual fear conditioning compared to control and pre-treated atropine significantly counteract the effect.

The data was statistically analyzed with one-way ANOVA followed byTukey post hoc test.

Data is demonstrated as mean ± SEM, ***p<0.001

a significant different from control

b significant different from pre-treated atropine.

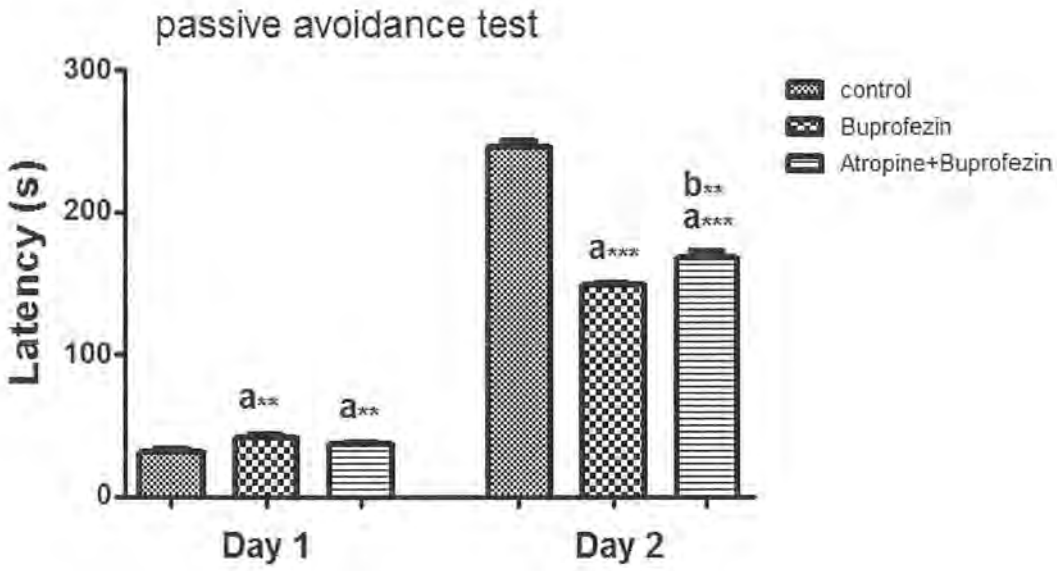


Fig 3.10.plot showed the acute effect of buprofezin and pre-treated atropine on passive avoidance of rats. Note buprofezin treated rat have impaired passive avoidance after electric shock at day 2 and was slightly counteracted by pre- treated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, ***p<0.001, **p<0.01

a significant different from control

b significant different from pre-treated atropine.

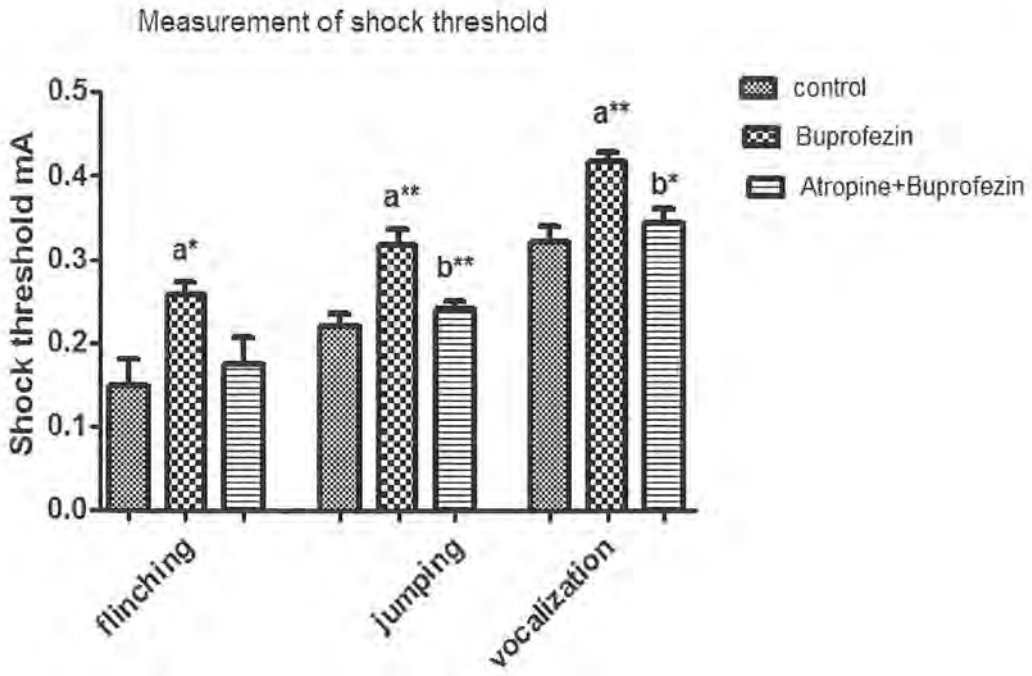


Fig 3.11.plot exhibit the quantification of shock threshold for flinching, jumping and vocalization in buprofezin treated and pre-treated atropine rats. Note the shock threshold for flinching, jumping and vocalization increased in buprofezin treated rats and is significantly counteracted by pre-treated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is presented as mean ± SEM, *$p<0.05$, **$p<0.01$

a significant different from control

b significant different from pre-treated atropine.

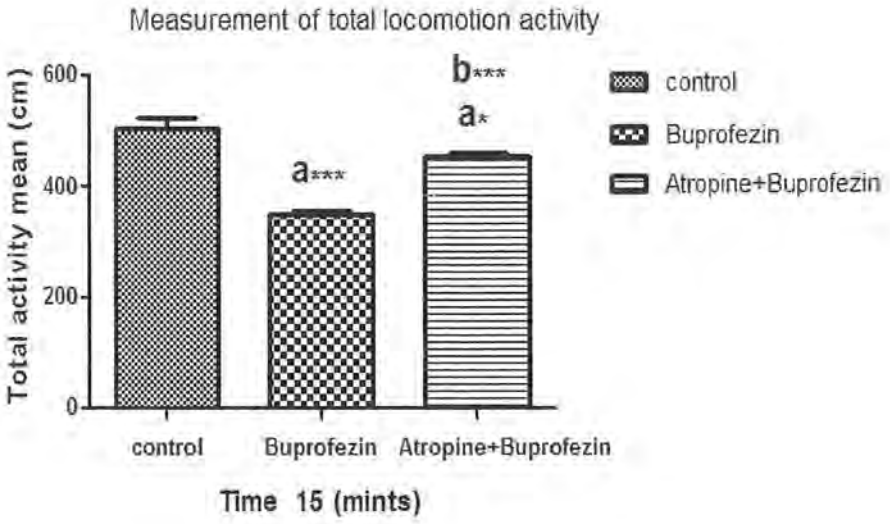


Fig 3.12.plot represent the effect of buprofezin and pre-treated atropine on total locomotion activity performed in TSD maze in 15 minutes. Note the significant decrease in locomotion activity by buprofezin administration is no significantly counteracted by pre-treated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *$p<0.05$, ***$p<0.001$

a significant different from control

b significant different from pre-treated atropine.

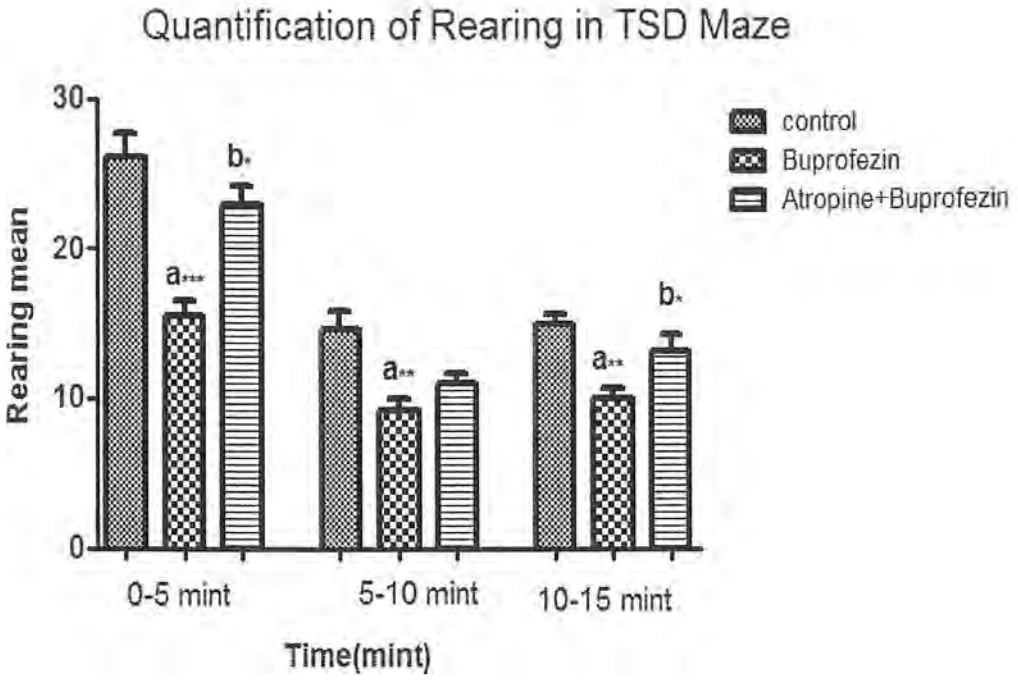


Fig 3.13.plot showed the number of rearing performed by rats in TSD maze for 15 minutes time duration. Note the buprofezin induced decrease in no of rearing is reversed by pre-treated atropine as no difference is observed in control and pre-treated atropine.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01***p<0.001

a significant different from control

b significant different from pre-treated atropine.

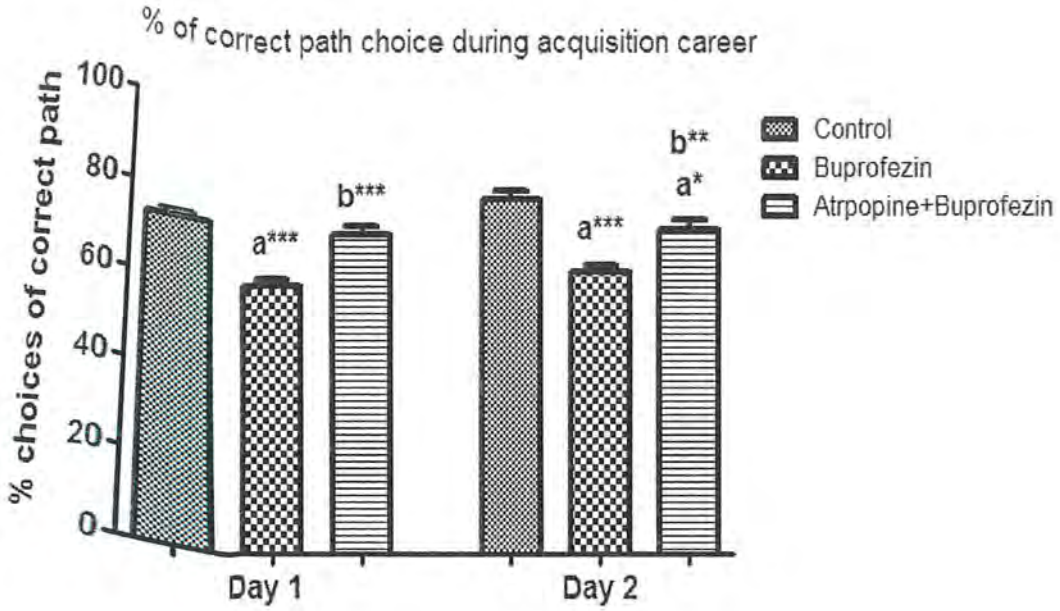


Fig 3.14.plot demonstrate the % of correct path choice during acquisition session and effect of buprofezin and pre-treated atropine on path selection by adult rats. Note the buprofezin treated rats exhibit less correctness of path selection as compared to control on day 1.Correctness of path selection is increased with training on day 2.pre-treated atropine have shown to increase % of correct path choice.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01***p<0.001

a significant different from control

b significant different from pre-treated atropine.

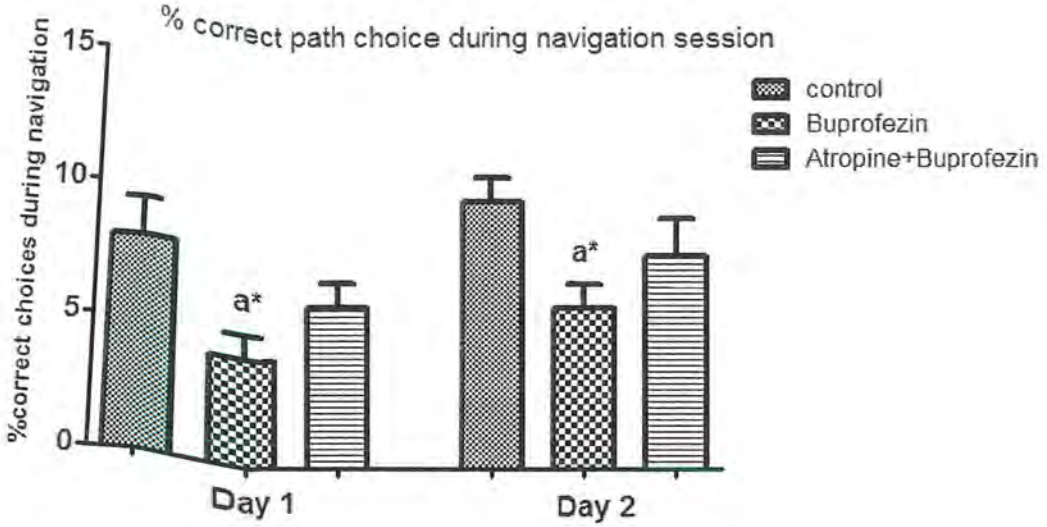


Fig 3.15.plot represent the % correctness of path choice during navigation session to achieve maximum 10% and effect of buprofezin and pretreated atropine on time space navigation of rats. Note buprofezin treatment significantly reduces the % correctness on day 1 and pre-treated atropine increases the % correctness during time space navigation. Training increases the % correctness as shown in day 2.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Values are presented as mean ± SEM, *p<0.05

a significant different from control

b significant different from pre-treated atropine.

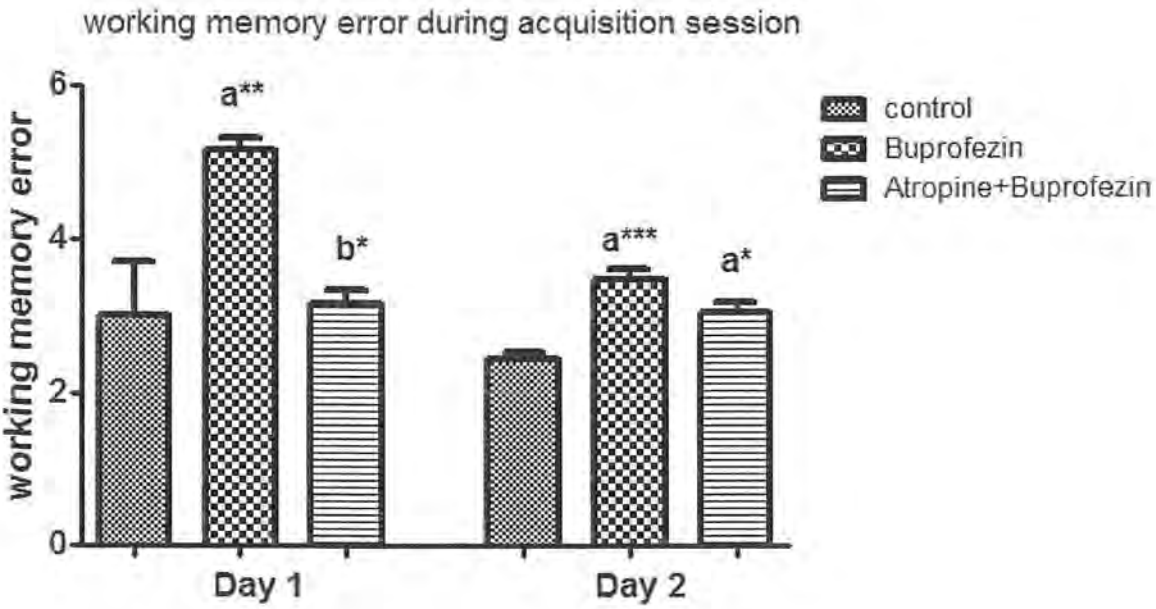


Fig 3.16.plot showed the effect of buprofezin and pre-treated atropine on working memory error (WME). Note the error has been increased after buprofezin exposure on day1 and is counteracted by pretreated atropine. On second day errors has been decreased because of training.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *p<0.05, **P<0.01***p<0.001

a significant different from control

b significant different from pre-treated atropine.

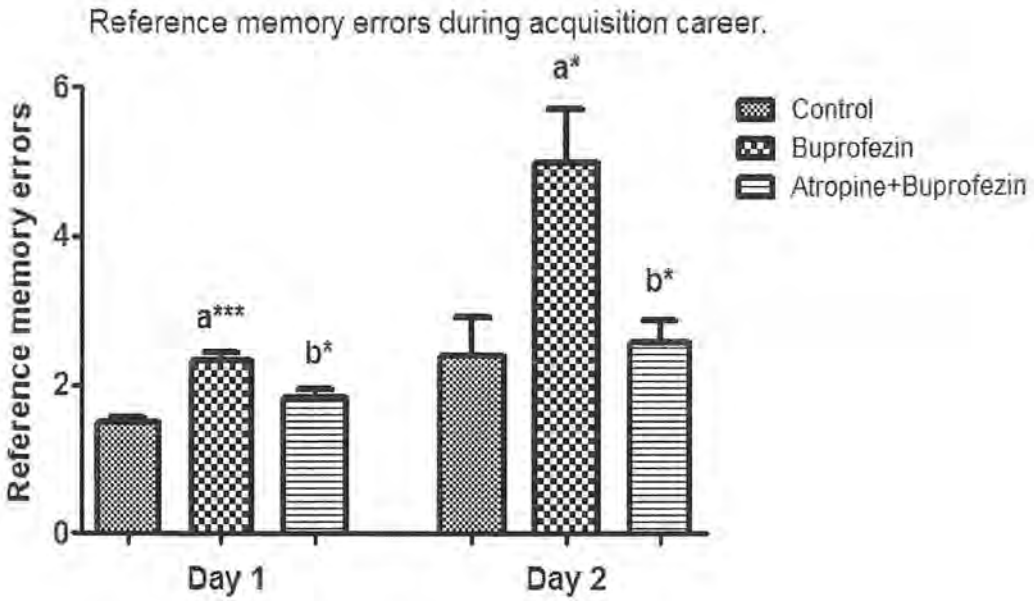


Fig 3.17. Plot showed the effect of buprofezin and pre-treated atropine on reference memory error (RME). Note the error are less after buprofezin exposure on day1 and is counteracted by pre-treated atropine. On second day errors has been increased and again decrease on successive days after training data not shown.

The data statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *p<0.05, ***p<0.001

a significant different from control

b significant different from pre-treated atropine.

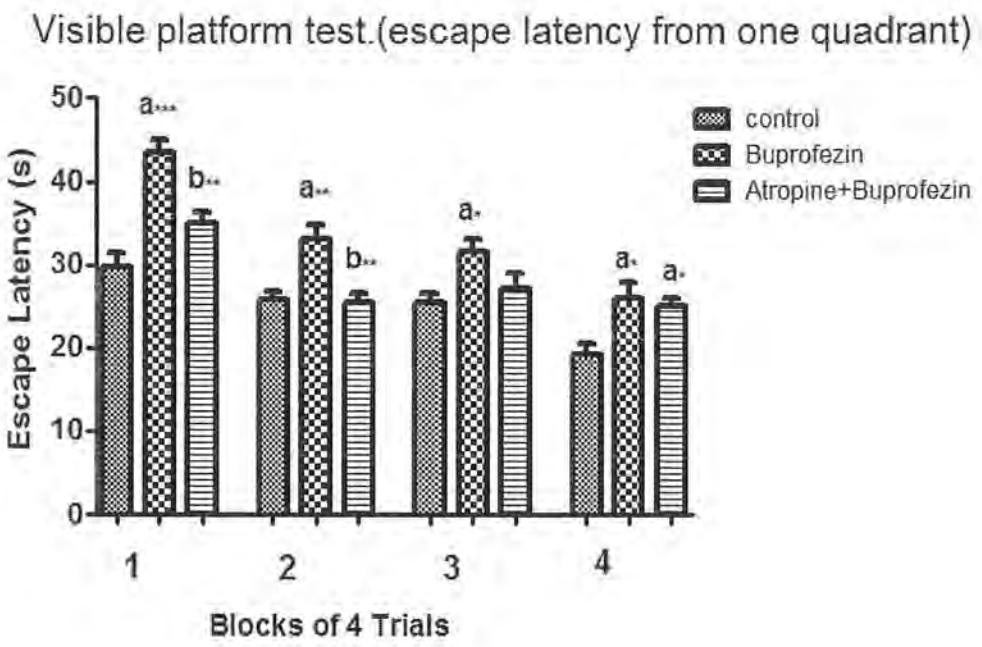


Fig 3.18. Plot showed the effect of buprofezin and pre-treated atropine on escape latency of rats from one quadrant of maze in visible platform test. Note the escape latency of buprofezin treated rats increases significantly compared to control and pre-treated atropine counteract. Training decrease the escape latency from quadrant.

The data statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, **p<0.01***p<0.001

a significant different from control

b significant different from pre-treated atropine.

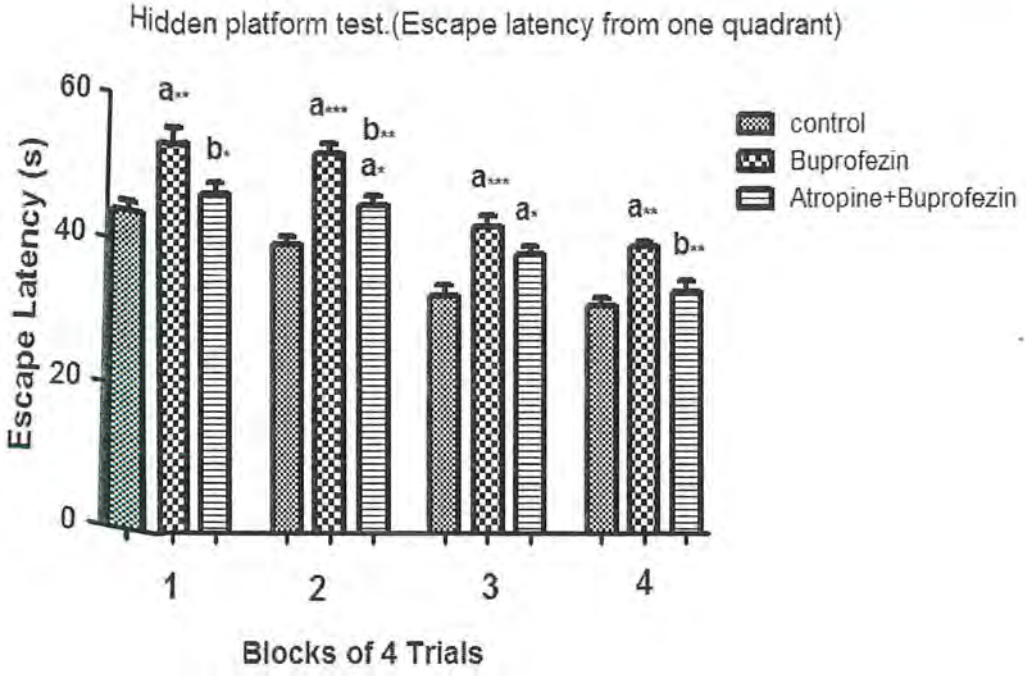


Fig 3.19. Plot showed the effect of buprofezin and pre-treated atropine on escape latency of rats from one quadrant of maze in hidden platform test. Note the escape latency of buprofezin treated rats increases significantly compared to control and pre-treated atropine counteract the effect. Training decrease the escape latency from quadrant.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *p<0.05, **p<0.01***p<0.001

a significant different from control

b significant different from pre-treated atropine.

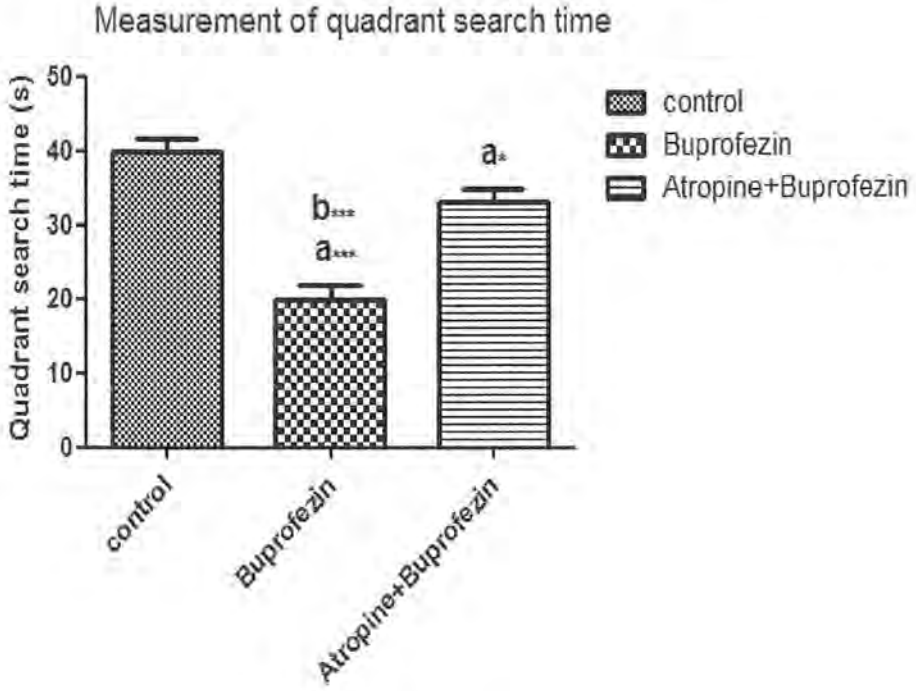


Fig 3.20.plot showed the quadrant search time in all treated groups during probe testing. Note the quadrant search time in buprofezin treated rats was significantly decreased compared to control. Pre- treated atropine attenuate the effect but still there is significant difference compared to control.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, ***p<0.001

a significant different from control

b significant different from pre-treated atropine.

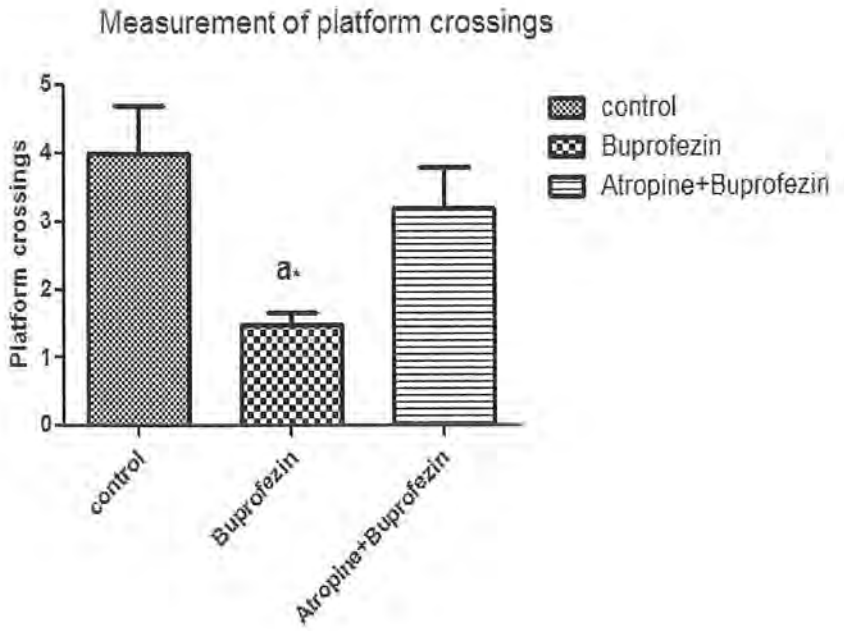


Fig 3.21.plot represent the effect of buprofezin and pre-treated atropine on number of platform crossings during probe testing. Note the significant decrease in platform crossings in buprofezin treated compared to control and pre-treated atropine has reversed the effect.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *$p<0.05$

a significant different from control

b significant different from pre-treated atropine.

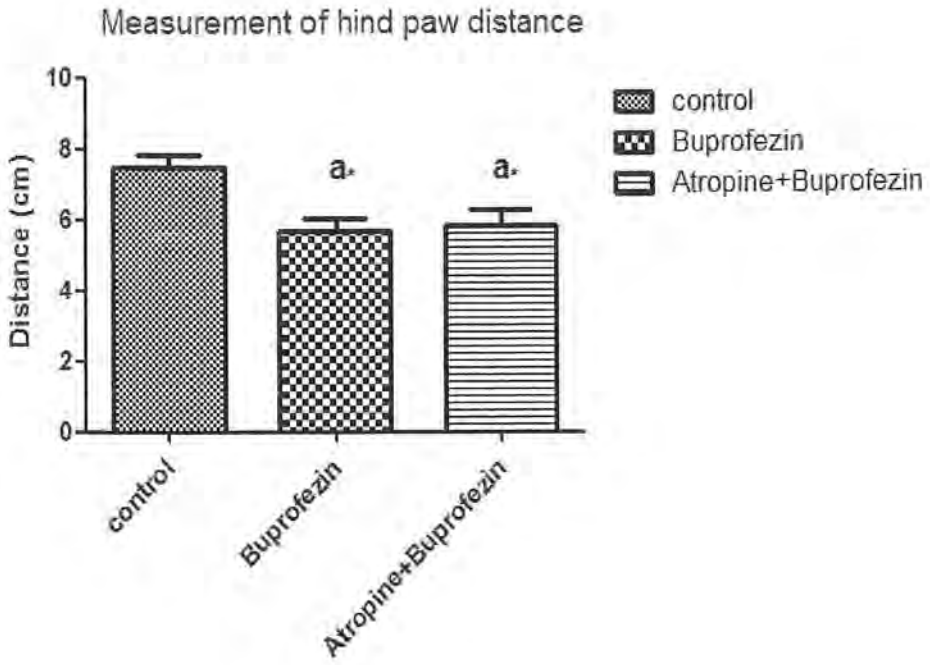


Fig 3.22.plot showed the effect of acute buprofezin exposure and pre-treated atropine on foot splay of rats. Note the significant decreased foot splay in buprofezin treated rats compared to control. Pre-treated atropine has slight counteract effect.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, *$p<0.05$

a significant different from control

b significant different from pre-treated atropine.

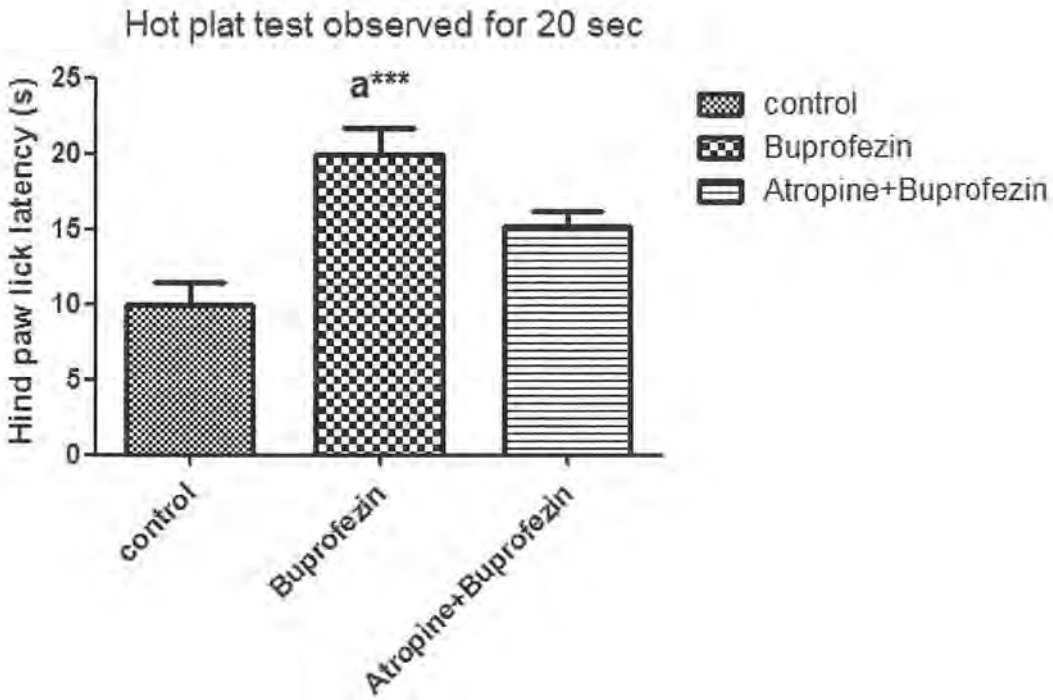


Fig 3.23. Plot presents the effect of acute buprofezin exposure and pre-treated atropine on nociception of rats. Note the significant increased hind paw lick latency in buprofezin treated rats compared to control. Pre-treated atropine has little counteract effect.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as mean ± SEM, ***p<0.001

a significant different from control

b significant different from pre-treated atropine.

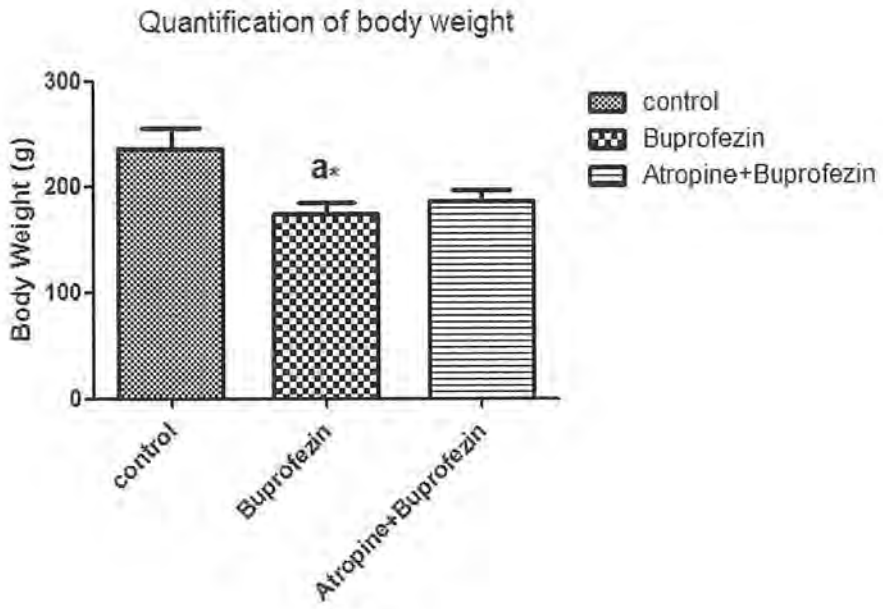


Fig 3.24. Plot present the effect of buprofezin and pre-treated atropine on body weight of rats. Note the body weight of buprofezin treated rats was significantly decreased as compared to control and pre-exposed atropine reversed this change.

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test.

Data is demonstrated as as mean ± SEM, *$p<0.05$

a significant different from control

b significant different from pre-treated atropine.

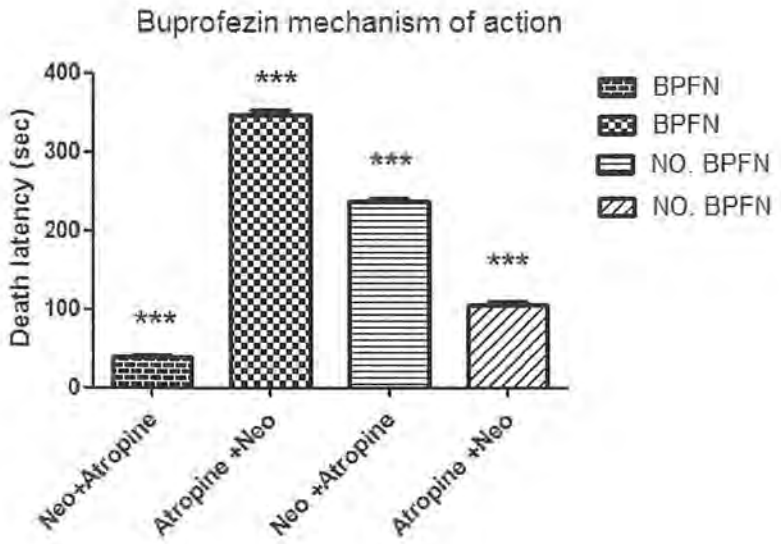


Fig 3.25. Showed the latency of death following the alternative exposure of neostigmine plus atropine in buprofezin intoxicated and non-intoxicated rats. P value <0.001.significant latency difference was exhibited by each group. Data was expressed as mean ±SEM.

Table 3.1. Showed the hematological data of acute buprofezin and pre-treated atropine plus buprofezin toxicity studies in Sprague-dawley male rats.

parameters	Units	Control Mean ± SEM	buprofezin Mean ± SEM	Atropine + buprofezin Mean ± SEM
White blood cell count (WBCs)	$10^3/\mu L$	8150 ± 571.63	**10552±507.14^{a*}**	**10810 ± 338.33^{a**}**
Lymphocytes (Ly)	%	38.4±4.37	**21.4 ± 4.06^{a*}**	**17.6 ± 2.56^{a**}**
Monocytes (Mo)	%	52.4 ± 4.97	**78 ± 4.64^{a*}**	**72.2 ± 3.52^{a*}**
Granulocytes (GR)	%	4.6 ± 1.21	4.2 ± 1.24	3.6 ± 1.43
Red blood cells (RBCs)	$10^6/\mu L$	5.94 ± 0.37	6.516 ± 0.17	6.524 ± 0.27
Hemoglobin (Hgb)	g/dL	11.88 ± 0.52	11.96 ± 0.15	12.42 ± 0.32
Hematocrit (HCT)	%	34.44 ± 1.63	35.54 ± 1.15	37.62 ± 1.68
Mean corpuscular cell volume (MCV)	fL	58.2 ± 0.99	55.16 ± 0.58	57.68 ± 0.93
Mean corpuscular Hb concentration	g/dL	34.54 ± 0.19	32.94 ± 0.99	33.14 ± 1.01
Red blood cells distribution width (RDW)	%	18.15 ± 1.14	**14.8 ± 1.14^{a*}**	**13.1 ± 0.74^{a*}**
Platelets (plt)	$10^3/\mu L$	530 ± 134.64	**987.8 ± 134.64^{a*}**	**1009.4 ± 120.82^{a*}**
Mean platelet cell volume (MPV)	fL	8.5 ± 0.61	7.86 ± 0.27	8.06 ± 0.32

The data was statistically analyzed with one-way ANOVA followed by Tukey post hoc test. Data is demonstrated as mean ± SEM, *p<0.05, **p<0.01

a significant different from control

b significant different from pre-treated atropine.

Table 3.2. List of parameters evaluated in FOB (functional observational battery) testing.

Parameters	Control	buprofezin	Atropine+buprofezin
Home cage observations			
Posture(D)	100	**80 ****	**90 ***
Vocalization (B)	0	0	0
Respiration (D)	100	100	100
Activity level (R)	1.0	**1.2 ***	1.1
Convulsions (D)	100	100	100
Hand held observations			
Lacrimation (R)	1.0	**1.2 ***	1.0
Salivation (R)	1.0	**1.2 ***	1.0
Piloerection (B)	0	**1 ***	0
Normal abdominal tone (D)	100	**80 ****	**90 ***
Limb grasping (B)	100	**80 ****	**90 ***
Close Maze observations			
Ambulation (D)	100	**80 ****	**90 ***
Gait (D)	100	**80 ****	**90 ***
Rearing (R)	0	**1***	0
Arousal (R)	0	**1***	0
Fecal boli count (C)	0	**1***	0
Involuntary motor movement (D)	1.0	1.0	1.0
Time space navigation (R)	0	**1***	0
Sensory observation			
Touch response (R)	2.0	**1.5 ***	**1.7 ***

Palpebral Reflex (B)	1.0	**1.2** *	1.0
Tail pinch response (R)	2.0	**1.5** *	**1.7** *
Pupil light response (R)	2.0	**1.5** *	**1.7** *
Physiological observations			
Body temperature (C)	1.0	**1.2** *	1.0
Body weight (C)	1.0	**1.2** *	1.0
Neuromuscular observations			
Limb tone (B)	100	**80** **	**90** *
Flexor reflex (B)	100	**80** **	**90** *
Extensor reflex (B)	100	**80** **	**90** *
Landing splay(C)	7.4	**5.7** *	**5.8** *
Righting Reflex (R)	1.0	**1.2** *	1.0

Descriptive (D) data is presented as percentage of incidence (Chi-square test) Binary (B) data is also demonstrated as percentage of incidence (Chi-square test). Ranked (R) data stated as the mean score of the scale used (Kruskal–Wallis test). Continuous (C) data stated as mean value (Two-way ANOVA test).

*$p < 0.05$ and ** $p < 0.01$ compared to control group

DISCUSSION

The recent study described the neurobehavioral toxicity of acute buprofezin exposure however it is previously well established that buprofezin (BPFN) frequently used as a moulting inhibitor insecticide all over the world to eradicate pests like leafhoppers, mealy bugs and whitefly (Chang et al., 2015), infiltrating the fruit crops, leafy crops and citrus crops. Its metabolic compounds are potentially hazardous to the neighboring milieu (EFSA, 2007). It has been proved extremely toxic to aquatic environment (EFSA, 2010: Ku et al., 2015) as in embryo of zebra fish reactive oxygen species (ROS) have been detected following the exposure to buprofezin and nickel. Administration of embryos and larvae of African catfish (Clarias gariepinus) to various doses (0, 0.05, .5, 5, 25, 50 and 100 mg/L) of buprofezin consequences into death of embryos when its amount of dose rises to 5–100 mg/L. In African catfish dose < 5 mg/L also carry out numerous hazardous effects during embryogenesis and development of larva. These effects comprises asymmetrical head, bleeding from pericardium, inward curvature of the lumbar and cervical regions, arcuate in body, ulcerates and accumulation of fluid in yolk sac (Marimuthu et al., 2013).

In our study we have explored that the acute oral dose of buprofezin 87.9 mg/kg/day induced wide range of neurobehavioral toxic effects. Acute intoxication of buprofezin induce a wide range of neurobehavioral toxicity including damage of pyramidal cells of hippocampal CA1, CA2 and CA3,region neurons and behavioral impairments for example, loss of motor coordination, locomotor activity, fear loss, hearing, heat shock, sensorimotor, cognitive and spatial navigation impairment following the acute exposure in adult Sprague dowley male rats. We have also found that acute intoxication of Buprofezin is potentially reversed by pre-administration of Atropine. The complete molecular and biochemical mechanism of Buprofezin neurobehavioral toxicity is not elucidated so for however we suggested that it inhibit the synthesis and release of AChE in synapse as in our experiment Buprofezin intoxicated rat was suddenly dead after the administration of neostigmine (30µl/kg/day i.p) a blocker of AChE. It put forward that a small concentration of AChE in synapse was dominantly occupied by a small concentration of neostigmine consequently tremendously elevated the ACh level in synapse leading to tremor and death of rats. This hypothesis was also supported by previous studies as activity of cytochrome and TCA cycle enzyme was rendered by

buprofezin that interfere the energy metabolism and inhibit production of ATP. (Ji et al., 2016; Binukumar et al., 2010; Shan et al., 2013).Atropine function as a physiological antagonist and competitively block the acetylcholine action of muscarinic receptors and acts as antidote for excessive parasympathetic activation arised as consequence of inhibition of AChE (Johnson et al., 2000). Acetylcholinesterase (AChE) being serine hydrolase enzyme that cleaves rapidly terminate the cholinergic transmission in synapse by breakdown of Ach into choline and acetate (Soreq and Seidman, 2001). AChE also described as key player in activation of glial cells, brain blood flow, amyloid pathway, phosphorylation of tau protein, also function as adhesion protein for maintenance and development of synapse (Ballard et al., 2005). Arsenic exposure in animal model induce behavioral alteration, abnormality in nervous system shaping and development, inflammation and neuron death, (Yen et al., 2011; Flora et al., 2012). In addition, arsenic could induce toxicity in HAPI microglia (Mao et al., 2016), granular neurons of cerebellum (Liu et al., 2013) and snail neurons (Lu et al., 2009).

Further our study demonstrated the impairment of motor coordination and fore limb and hind limb grip strength as Occupational exposure to acrylamide leads to cumulative but reversible neurotoxicity described by axanopathy of peripheral nerves, ataxia, muscle weakness, tingling of hands and feet and cognitive deficiency. Because it inhibit kinesin transport, decrease the neurotransmitters and inhibition of transmission (Exon, 2006). Crofton and colleagues stated decreased grip strength in a 30 day acrylamide administration study at 15 and 20 mg/kg/days because it cause peripheral axonopathy (Crofton et al., 1996) after exposure to high oral doses of buprofezin clinical signs appear as reduced locomotor activity, tremble, runny nose, abnormal movement and urinary discontinuous urination. (*EFSA*, 2007). In present study impairment in the grip strength and motor coordination was assessed by using rotarod, horizontal and parallel bars. Similar impairments in motor activity, abnormal gait, and cognitive deficiency were detected following exposure to bifenthrin due to oxidative stress (Farah et al., 2017) A study on earthworm described that reduction in growth rate by combined exposure of buprofezin, Lufenuron, and Triflumuron pesticide-exposed worms was observed by dose-dependent over the 28-day treated duration, which was accompanied by a decline in activity of AChE and GST. The lowest activity of AChE was noted at the highest dose of buprofezin (300 mg/kg soil) following two weeks of exposure as

compared to control. The activity of AChE was intensely repressed by lufenuron subsequently by buprofezin, and then triflumuron in descendant. (Badawy et al., 2013).Similarly our study showed that acute exposure of buprofezin inhibit the synthesis and release of AChE in synapse due to limited supply of ATP described by other study that buprofezin efficiently repressed the cytochrome c oxidase activity by binding to SCO1 active pockets and COX17, which increased the concentration of reactive oxygen species. Additionally, administration with an ROS inhibitor (N-acetyl-L-cysteine) (NAC) counteract the decreased level of ATP and cytochrome c oxidase activity, which also showed that ROS contributed in buprofezin-induced conversion of energy metabolism. After sub lethal treatment of buprofezin, the levels of the end product metabolism (ATP), end product of glycolysis (lactate) and (pyruvate) a component in initial stage of the TCA cycle were evaluated. The higher concentration of these factors after buprofezin exposure reveals the BPFN induced inhibition of TCA cycle. Pesticides can decreased the ATP concentration in HepG2 cells revealed by in vivo and in vitro studies (Binukumar et al., 2010; Shan et al., 2013).

Pre- treated atropine counteract the poisoning by blocking muscarinic receptors and it counteracted over parasympathetic activity. At single oral dose (24 h) of chlorpyrifos reduced the activity of plasma butyrylcholinesterase (BChE) and rats (AChE) activity in hippocampus, striatum and prefrontal cortex. The acute chlorpyrifos toxicity can be counteracted by the atropine antidote (10 mg/kg i.p.) and/or pralidoxime (40 mg/kg; i.p.) treated one hour following toxicity (Alciene Almeida Siqueira et al., 2019). In pre-clinical and clinical experiments muscarinic antagonists exhibit antidepressant effects (Drevets et al., 2013; Mancinelliet al., 1988; Witkin et al., 2014). Acute buprofezin decreases the synthesis and release of AChE in synapse. Furthermore (ip) neostigmine injection in buprofezin intoxicated rats cause tremor, and sudden death of rat also proposed that minor amount of AChE is predominately blocked by neostigmine extremely elevated the ACh level in synapse subsequent acute exposure and is counteracted by pre-treated atropine.

Buprofezin exposure impair the passive avoidance as MSK1 knockout affects numerous various forms of hippocampus-dependent memory, as evaluated by fear conditioning, Morris water maze and passive avoidance (Wilson et al.,2007). Pre-treated atropine showed counteract effect not reported in any previous study.

The behavioral analysis revealed that SA (sodium arsenide) treatment cause loss in learning and memory in passive avoidance as well as motor activity and balance. Additionally acute or chronic administration to SA as revealed by other experiments induce abnormalities of CNS containing slowing of cognitive development, decreased psychomotor speed, loss of learning due to decreased number and apoptosis of pyramidal cells and was mitigated by ellagic acid. (Franzblau et al., 1989; Mathew et al., 2010; Tsai et al., 2003) .Our finding also agreed with similar behavior abnormalities and loss of passive avoidance learning and memory due to pyramidal neuron damage in hippocampus and was attenuated by pre –treated atropine. Buprofezin induced decreased in the step- through latency was reversed by pre- treated atropine as the SA exposure has showed significant reduction in the step-through latency comparison to the control due to oxidative stress (Mehdi et al., 2018). The study reported that MSK1 knock-out animals can process the sensory information of foot shock and memorize it in association with contextual and the auditory stimulus, but loss this memory in 24h. (Wilson et al 2007). Our study reported the impairment in long tern contextual fear memory and rapid loss of sensory stimulus of foot shock in acute buprofezin exposure and was slightly counteracted by pre-treated atropine.

Various concentration of orally administered imidacloprid to female rats caused a substantial change in different features of locomotor activity and decreased in ambulation at 90 days of treatment as it inhibit AChE activity (Shipra et al., 2010).Substantial reduction in locomotion in the rats exposed with the acute dose of imidacloprid has suggested that imidacloprid or its metabolic residues has accumulated in brain. Administration of imidacloprid directly in to intraperitonium has shown to accumulated in mouse brain (Chao et al., 1997).

Although the mechanism of action of buprofezin is distinct to some extant results described the similar decline in spontaneous locomotion activity in acute buprofezin administered rats compared to control and pre-treated atropine counteract the toxicity. Similar to high dose of imadacloprid decreases the spontaneous locomotor activity. Another study also supported our results that during the peak of the BGS (brain growth spurt) (PND10) administration of single dose of endosulfan or cypermethrin, cause long lasting spontaneous behavior abnormality in adults due to alteration of protein involved in brain development, variation in locomotion, rearing and total activity without affecting body weight as compared to control mice (Lee et al., 2015).

It has also been stated that the number of hippocampal neurons declined in rats after treatment with sulfite (Akdogan et al., 2011). Additionally, it was revealed that after sulfite exposure the excitability of the spinal reflexes was increased (Küçükatay et al., 2005; Küçükatay et al., 2008). The toxic consequences of sulfite on mesencephalic cell lines have been described, as well (Reist et al., 1998).

Our study in line with these findings as pre-treated atropine has neuroprotective effect against buprofezin toxicity. Pre-treated atropine prevent hippocampal neuron degeneration, spatial memory impairment, working and reference memory loss by preventing over excitability induced neuron exhaustion and preventing the apoptosis of hippocampal neurons. It also prevent the oxidative stress of hippocampal neurons and protect against buprofezin induced cognitive impairment as curcumin inhibit lead-induced loss of memory in rats (Dairam et al., 2007). Curcumin can alleviate the cognitive deficit in diabetic rats (Kuhad et al 2007).Therefore, curcumin may preclude the oxidative stress in CA neurons and, as result, may enhance the synaptic plasticity (Noorafshan et al., 2013; Kuhad et al 2007). It is also stated that curcumin protect the neurodegeneration in Parkinson and Alzheimer disease. (Yadav et al., 2009). Additionally, it is also suggested that curcumin prevent the apoptosis of neuron. (Lin et al., 2011).

However, an impairment of spatial memory ability was observed in 1- month- rotenone exposed group model animal. Later it was well acknowledged that the hippocampus was indispensable for spatial learning and memory performance on Morris water maze task (Martin et al., 2007: Ryan et al., 2010). Earliest data demonstrate that ISS produces an impairement in MWM task (unpublished results). Yet, ISS-induced impairement in the forced swim test is potentially attenuated by NSRIs. (Drugan et al., 2010). Consequently, NSRIs, desipramine (Norpramin) and reboxetine (Edronax), potentially reverse the stress induced deficit in the forced swimming test. (Drugan et al., 2010) and reboxetine (Cryan et al., 2002; Page and Lucki, 2002). The previous study described that (3.5 mg kg-1 and 7 mg kg-1) doses of bifenthrin caused remarkable spatial memory deficit of the rats hence the quadrant spending time was significantly less compared to control. (Farah et al., 2017).In contrary in our findings buprofezin intoxicated rats exhibit increased escape latency compared to control. Additionally quadrant search time and numbers of platform crossings were decreased compared to control.

Yet, another study described Hind limb foot splay of both males and females assessed on the day 15 exhibited statistically significant increase in male rats exposed with acrylamide at 40 mg/kg body weight as compared to control and showed no effect on female.. In an earlier study, acrylamide at 40 mg/kg represented a biologically substantial increase in foot splay. (Patil et al., 2015). In contrary our study revealed the significant decrease in hind limb foot splay distance in acute buprofezin treated rats compared to control and pre-treated atropine counteract the effect. These finding suggested that pre-treated atropine attenuate the peripheral neuropathy caused by acute exposure of buprofezin.

Our results also demonstrate that acute exposure of buprofezin produce anti nociceptive effect and is reversed by pre-treated atropine as acute exposure of complex PC/β-CD (p-cymene/β-cyclodextrin (β-CD) complex induce an anti-nociceptive effect for 8 h later whereas only PC induce the similar effect for 2 h. Parallel results were found with treatment of PC/β-CD in hot-plate test, for all doses, remarkably decreased the sense of nociception for 8 h while only PC for 1 h, occur only at high doses likely caused by motor abnormality(Jullyana de Souza Siqueira Quintans et al., 2013).

The finding of our study reported that acute oral administration of buprofezin cause weight loss and decrease the food consumption. A small weight gain was noticed in pre-treated atropine rats as demonstrated that oral administration of buprofezin 73.97 mg/kg bw/day in males and 93.11 mg/kg bw/day in females enhance the weight of organs and decreased the body weight gain (Toyohara, 1997). It is also described that administration of 200 ppm (17 mg/kg bw day male; 20.5 mg/kg bw females) decreased food intake and biochemical factors (Watanabe, 1986).

Hematological parameters were demonstrated that the WBC, lymphocyte and monocyte count was significantly high in chlorpyrifos (CPF)-treated models but significantly lower counts of granulocyte compared to control animals. The animal exposed to CPF plus GSH (*Glutathione*)- revealed a significant increase in lymphocytes and substantial decrease in granulocytes as compared to control as (CPF) cause by oxidative stress. (Eman et al., 2013) Our results agreed with these previous finding as there was significant increase in white blood cells (WBCs), lymphocytes, monocytes, red blood cells distribution width and platelets. Pre-treated atropine showed no significant effect on reversal.

A wide range of effects produced by Trichlorethane (TCE), and ether are similar to ethanol and depressant like properties of volatile solvent described in earlier literature (Evan et al.,1996) these involved decreases in activity of CNS (alterations in posture. reduced arousal and rearing), decreases in emotionality of CNS (increased ease of removal), impair of muscle tone (disturbances in gait reduction in forelimb grip strength, increased landing foot splay. and loss of psychomotor coordination on the inverted screen test), and decreased sensorimotor activity (reduced response to sensory stimuli).Though TCE and ether at concentrations of 13.300 and 30.000 ppm substantially increased landing foot splay. Flurothyl exposure did not affect increase in landing foot splay at any concentration. TCE and ether also decrease forelimb grip strength but not flurothyl. Additionally flurothyl produced handling-induced tremors after after animals was removed from cage, effect which was not caused by TCE, ether or ethanol. Flurothyl cause postictal depression (e.g. an increased inversion latency on the inverted screen test) Characterized by convulsion in testing animal and exhibit not any ethanol like properties (Scott e et al., 1996). The results of our study also agreed with the with these findings as the acute exposure of buprofezin in rat results in decreased brain activity, posture abnormality, decreased activity level, increased lacrimation, salivation, piloerection, abnormal muscle tone, loss of limb grip, ambulation and gait abnormalities, decreased rearing and arousal, loss of time space navigation ability, loss of sensorimotor responses, Redness of nostrils, muscle hypoplasia, decrease in body weight and body temperature. However pre-treated atropine attenuated these variation and significantly reversed these effects.

The farmer studies have various shortcoming as they devoid acute buprofezin neurotoxicity in rat model. Additionally none of previous study has reported the mechanism underlying its neurotoxicity in rats. Further no therapeutic strategy against buprofezin toxicity was carried out. In contrary our finding have proved that acute exposure of buprofezin induce wide range of neurobehavioral toxicity in adult Sprague dowely male rats and potential antidote against its neurotoxicity

With the advancing incidents of neurobehavioral and cognitive defects the objective of this study was to investigate the neurobehavioral toxicity, underlying mechanism, and possible therapeutic approach neurobehavioral to overcome its neurotoxicity following acute exposure of Buprofezin. In conclusion our findings have explored that acute exposure of buprofezin in rats induces a profile of neurobehavioral toxicities potentially

attenuated by pre-treated atropine antidote. Moreover its neurobehavioral toxicity was induced by impairment of synthesis and release of acetylcholinesterase (AChE) in synapse. Further studies are necessary to validate molecular and biochemical mechanism involved in decrease concentration of AChE in synapse.

References

Akdogan I, Kocamaz E, Kucukatay V, Yonguc NG, Ozdemir MB, Murk W. (2011). Hippocampal neuron number loss in rats exposed to ingested sulfite. Toxicol Ind Health 27:771-778

Akdogan I, Kocamaz E, Kucukatay V, Yonguc NG, Ozdemir MB, Murk W.(2011). Hippocampal neuron number loss in rats exposed to ingested sulfite. *Toxicol Ind Health* 27:771-778

Alciene Almeida Siqueira, Alexandre Frinhani Cunha, Graziany Leite Moreira Marques,Igor Simões Assunção Felippe, Vitor Sampaio Minassa, Thallis Coelho da Silva Gramelich Maria Aparecida Cicilinic, Tamara Andrea Alarcon, Rita Gomes Wanderley Pires Karla Nivea Sampaio Vanessa Beijamin .(2019). Atropine counteracts the depressive-like behaviour elicited by acute exposure to commercial chlorpyrifos in rats. Neurotoxicology and Teratology 71:6-15

Ali Noorafshan, Reza Asadi-Golshan, Saied Karbalay-Doust, Mohammad Amin Abdollahifar, and Ali Rashidiani-Rashidabadi.(2013). Curcumin, the Main Part of Turmeric, Prevents Learning and Memory Changes Induced by Sodium Metabisulfite, a Preservative Agent, in Rats. *Exp Neurobiol.* 22(1):23-30

Ali Noorafshan, Reza Asadi-Golshan, Saied Karbalay-Doust, Mohammad Amin Abdollahifar, and Ali Rashidiani-Rashidabadi.(2013). Curcumin, the Main Part of Turmeric, Prevents Learning and Memory Changes Induced by Sodium Metabisulfite, a Preservative Agent, in Rats. Exp Neurobiol. 22(1):23-30

Arnold, D. L., Charbonneau, S. M., Zawidzka, Z. Z., and Grice, H. C. (1977). Monitoring animal health during chronic toxicity studies. J Environ Pathol Toxicol 1: 227–39.

B Weiss, V G Laties. (1969). Behavioral Pharmacology and Toxicology. 1969(9):297-326

B. Binukumar, A. Bal, A. Sunkaria, K.D. Gill. (2010). Mitochondrial energy metabolism impairment and liver dysfunction following chronic exposure to dichlorvos, Toxicology 270: 77–84

Balaban, C. D. (1985). Central neurotoxic effects of intraperitoneally administered 3-acetylpyridine, harmaline and niacinamide in Sprague-Dawley and Long-Evans rats: A critical review of central 3-acetylpyridine neurotoxicity. Brain Res 356:21–42.

Barnes, J. M., and Denz, F. A. (1954). Experimental methods in determining chronic toxicity: A critical review. Pharmacol Rev 6:191–242.

Bernard Weiss., 1988. Behavior as an Early Indicator of Pesticide Toxicity *Toxicology and Industrial Health*, 4:351-360.

Bertelli, J. A., and Mira, J. C. (1995). The grasping test: A simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods 58: 151–55.

Biel, W. C. (1940). Early age differences in maze performance in the albino rat.

Brown, A. W., Aldridge, W. N., Street, B. W., and Verschoyle, R. D. (1979). The behavioral and neuropathologic sequelae of intoxication by trimethyltin compounds in the rat. Am J Pathol 97: 59–82.

Broxup, B., Robinson, K., Losos, G., and Beyrouty, P. (1989). Correlation between behavioral and pathological changes in the evaluation of neurotoxicity. Toxicol Appl Pharmacol 101: 510–20.

Butterworth, R. F., Hamel, E., Landreville, F., and Barbeau, A. (1978). Cerebellar ataxia produced by 3-acetyl pyridine in rat. Can J Neurol Sci 5:131–33.

C.G. Ballard, N.H. Greig, A.L. Guillozet Bongaarts, A. Enz, S.Darvesh., (2005). Cholinesterases: roles in the brain during health and disease Curr. *Alzheimer Res.*, 2 307-318.

Cahill, L., McGaugh, J. L., and Weinberger, N. M. (2001). The neurobiology of learning and memory: Some reminders to remember. Trends Neurosci 24:578–81.

Calton, J. L., and Taube, J. S. (2009). Where am I and how will I get there from here? A role for posterior parietal cortex in the integration of spatial information and route planning. Neurobiol Learn Mem 91: 186–96.

Chang, X., Yuan, Y., Zhang, T., Wang, D., Du, X., Wu, X., Chen, H., Chen, Y., Jiao, Y., Teng, H.(2015). The toxicity and detoxifying mechanism of cycloxaprid and buprofezin in controlling sogatella furcifera (Homoptera: Delphacidae). *J. Insect Sci.* 15:1–5.–2537.

Chao Li, Ji Zhang, Zhi-Guo Wu, Li Cao, XinYan ,Shun-Peng Li.(2012). Biodegradation of buprofezin by *Rhodococcus* sp. Strain YL-1 Isolated from Rice Field Soil *J. Agric. Food Chem.* 60:2531-2537.

Chao, S.L., Casida, J.E. (1997). Interaction of imidacloprid metabolites and analogs with the nicotinic acetylcholine receptor of mouse brain in relation to toxicity. Pest. Biochem. Physiol. 58, 77–88.

Cottage, Emma L A; Gunning, Robin V. (2006). buprofezin inhibits acetylcholinesterase activity in B-biotype Bemisia tabaci. *WorldWideScience.org.*

Crawley, J. N. (1999). Behavioral phenotyping of transgenic and knockout mice: Experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. Brain Res 835:18–26.

Crawley, J. N. (2003). Behavioral phenotyping of rodents. Comp Med 53: 140–46.

Crawley, J. N., and Paylor, R. (1997). A proposed test battery and constellations of specific behavioral paradigms to investigate the behavioral phenotypes of transgenic and knockout mice. Horm Behav 31:197–211.

Crofton KM, Padilla S, Tilson HA, Anthony DC, Raymer JH, Macphail RC. (1996). The impact of dose rate on the neurotoxicity of acrylamide: the

interaction of administered dose, target tissue concentrations, tissue damage, and functional effects. *Toxicology and Applied Pharmacology*.139:163–176.

Crofton, K. M. (1990). Reflex modification and the detection of toxicantinduced auditory dysfunction. Neurotoxicol Teratol 12: 461–68.

Crofton, K. M., Dean, K. F., Hamrick, R. C., and Boyes, W. K. (1991). The effects of 2,4-dithiobiuret on sensory and motor function. Fundam Appl Toxicol 16:469–81.

Crofton, K. M., Howard, J. L., Moser, V. C., Gill, M. W., Reiter, L. W., Tilson, H. A., and MacPhail, R. C. (1991). Interlaboratory comparison of motor activity experiments: Implications for neurotoxicological assessments. Neurotoxicol Teratol 13:599–609.

Cryan JF, Page ME, Lucki I. (2002). Noradrenergic lesions differentially alter the antidepressantlike effects of reboxetine in a modified forced swim test. Eur J Pharmacol .436: 197–205.

D'Hooge, R., and DeDeyn, P. P. (2001). Applications of the Morris water maze in the study of learning and memory. Brain Res Rev 36: 60–90.

Dairam A, Limson JL, Watkins GM, Antunes E, Daya S. (2007). Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. J Agric Food Chem 55:1039-1044

Dairam A, Limson JL, Watkins GM, Antunes E, Daya S.(2007). Curcuminoids, curcumin, and demethoxycurcumin reduce lead-induced memory deficits in male Wistar rats. *J Agric Food Chem* 55:1039-1044

Dani C, Vestri V, Bertini G, Pratesi S, Rubaltelli FF. (2007). Toxicity of corticosteroids and catecholamines for mice neuronal cell cultures: Role of preservatives. J Matern Fetal Neonatal Med 20:325-333.

David M. Compton H. Randall Griffith William F. McDaniel Robert A. Foster Brenda K. Davis. (1997). The Flexible Use of Multiple Cue

Relationships in Spatial Navigation: A Comparison of Water Maze Performance Following Hippocampal, Medial Septal, Prefrontal Cortex, or Posterior Parietal Cortex Lesions. *Neurobiology of Learning and Memory* 68:117-132.

Davis, M., Gendelman, Tischler, Gendelman, and P. M. (1982). A primary acoustic startle circuit: Lesion and stimulation studies. J Neurosci 2: 791–805.

De Cock and D. Degheele buprofezin. (1998).A Novel Chitin Synthesis Inhibitor Affecting Specifically Planthoppers. Whiteflies and Scale Insects 74-91.

Derek C. Rogers E.M.C. Fisher S.D.M. Brown J. Peters A.J. Hunter J.E. Martin. (1997). Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. *Mammalian Genome* 8: 711–713.

Desclin, J. C., and Escubi, J. (1974). Effects of 3-acetylpyridine on the central nervous system of the rat, as demonstrated by silver methods. Brain Res 77:349–64.

Drevets, W.C., Zarate, C.A., Furey, M.L. (2013). Antidepressant effects of the muscarinic cholinergic receptor antagonist scopolamine: a review. Biol. Psychiatry 73, 1156–1163

Drugan RC, Macomber H, Warner TA. (2010). Intermittent and continuous swim stress induced behavioral depression: sensitivity to norepinephrine- and serotonin selective antidepressants. Psychopharmacology.212:85–91.

Dunham, N. W., and Miya, T. S. (1957). A note on a simple apparatus for detecting neurological deficit in mice and rats. J Am Pharm Assoc 46: 208–9.

Dyer, K. R., Jortner, B. S., Shell, L. G., and Ehrich, M. (1992). Comparative dose-response studies of organophosphorus ester-induced delayed neuropathy in rats and hens administered mipafox. Neurotoxicol 13:745–55.

Eaton, R. B., Daroff, H., Autrup, J., Bridges, P., Buffler, L. G., Costa, J., et al. (2008). Review of the toxicology of chlorpyrifos with an emphasis on human exposure and neurodevelopment. *Crit. Rev. Toxicol.* 38:1–125.

Edwards, P. M., and Parker, V. H. (1977). A simple, sensitive, and objective method for early assessment of acrylamide neuropathy in rats. Toxicol Appl Pharmacol 40:589–91.

EFSA (European Food Safety Authority) (2007). Opinion on genotoxic and carcinogenic potential of buprofezin in the context of the human risk assessment – scientific opinion of the panel on plant protection products and their residues (PPR). *EFSA J.* 620:1–28

EFSA (European Food Safety Authority). (2010). Conclusion on the peer review of thepesticide risk assessment of the active substance buprofezin. EFSA J. 8 (6), 1–77.

EFSA. (European Food Safety Authority). (2010). Conclusion on the peer review of thepesticide risk assessment of the active substance buprofezin. *EFSA J.* 8 (6), 1–77.

EFSA. (2013). The 2010 European Union Report on pesticide residues in food. EFSA J.3130, 808

Ehman, K. D., and Moser, V. C. (2006). Evaluation of cognitive function in weanling rats: a review of methods suitable for chemical screening. Neurotoxicol Teratol 28: 144–61.

Elizabeth A. Lochry Connie Johnson Patrick J. Wier. (1994). Behavioral evaluations in developmental toxicity testing: MARTA survey results. *Neurotoxicology and Teratology* 16:55-63.

Elizabeth Dean, P.T. Jocelyn Ross, B.S.R. Jeremy D. Road , Laurel Courtenay (P.T. Kim J. Madill. (1991). Pulmonary Function in Individuals with a History of Poliomyelitis. *Chest* 100: 118-12.

Eman E.Elsharkawy DohaYahia Neveen A.El-Nisr. (2013). Sub-chronic exposure to chlorpyrifos induces hematological, metabolic disorders and oxidative stress in rat: Attenuation by glutathione. 35(2)218-227

Evans. E. B.: Balster. R. L. (1991). CNS depressant effects of volatile organic solvents. Neurosci. Biobehav. Rev. 15:233-241

Exon JH, A review of the toxicology of acrylamide. (2006). *Journal of Toxicology and Environmental Health, Part B.* 93:97-412

Farah Syed, Kumud K. Awasthi, Lalit P. Chandravanshi, Rajbala Verma, Neelu K. Rajawat, Vinay K. Khanna,c P. J. Johna and Inderpal Soni.(2017). Bifenthrin -induced neurotoxicity in rats: involvement of oxidative stress. Published on 12 October 2017. Downloaded by University of Newcastle. 14:52:04.

Flora, S. J., Mittal, M., Pachauri, V., and Dwivedi, N. (2012). A possible mechanism for combined arsenic and fluoride induced cellular and DNA damage in mice. *Metallomics* **4**:78-90.

Fox, J. G. (1977). Clinical assessment of laboratory rodents on long term bioassay studies. J Environ Pathol Toxicol 1: 199–226.

Francis, Zaharia , Shanks N, Anisman H. (1995). Stress-induced disturbances in Morris water- maze performance: inters train variability. Physiol Behav. 58(1):57–65

Franzblau, R. Lilis. (1989). Acute arsenic intoxication from environmental arsenic exposure, Arch. Environ. Health 44: 385–390

G. Shan, M. Ye, B. Zhu, L. Zhu. (2013). Enhanced cytotoxicity of pentachlorophenol by perfluorooctane sulfonate or perfluorooctanoic acid in HepG2 cells, Chemosphere 93: 2101–2107

Gad, S. C., and Gad, S. E. (2003). A functional observational battery for use in canine toxicity studies: development and validation. Int J Toxicol 22:415–22.

Garth Nicholson Simon Myers. (2006). Intermediate forms of Charcot-Marie-Tooth neuropathy *Neuro Molecular Medicine.* 8: 123–130.

Gauvin, D. V., and Baird, T. J. (2008). A functional observational battery in non-human primates for regulatory-required neuro behavioral assessments. J Pharmacol Toxicol Methods 58:88–93.

Gerald, M. C., and Gupta, T. K. (1977). The effects of amphetamine isomers on rotarod performance. Psychopharmacol (Berl) 55: 83–86.

Gerlai, R. (2001). Behavioral tests of hippocampal function: Simple paradigms complex problems. Behav Brain Res 125:269–77.

Gilbert, S. G., and Maurissen, J. P. (1982). Assessment of the effects of acrylamide, methylmercury, and 2,5-hexanedione on motor functions in mice.J Toxicol Environ Health 10:31–41.

H. Sińczuk-Walczak. (2009). Nervous system disorders induced by occupational exposure toarsenic and its inorganic compounds: a literature review, Med. Pr. 60: 519–522.

H. Soreq, S. Seidman. (2001). Acetylcholinesterase – new roles for an old actor *Nat. Rev. Neurosci.* 2:294-302.

Hamm, R. J., Pike, B. R., O'Dell, D. M., Lyeth, B. G., and Jenkins, L. W. (1994). The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. *J Neurotrauma* 11:187–96.

Hamm, R. J., Pike, B. R., O'Dell, D. M., Lyeth, B. G., and Jenkins, L. W. (1994). The rotarod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J Neurotrauma 11:187–96.

Hayatsu H, Miller RC Jr. (1972). The cleavage of DNA by the oxygen-dependent reaction of bisulfte. Biochem Biophys Res Commun 46:120-124.

Henrik Selin. (2014). Global Environmental Law and Treaty-Making on Hazardous Substances: The Minamata Convention and Mercury Abatemen14, 1-19.

Hongna Sun1, Yanmei Yang1, Hanwen Shao1, Weiwei Sun1, Muyu Gu1, Hui Wang1, Lixin Jiang1, Lisha Qu1, Dianjun Sun1 and Yanhui Gao. (2017). Sodium Arsenite-Induced Learning and Memory Impairment Is

Associated with Endoplasmic Reticulum Stress-Mediated Apoptosis in Rat Hippocampus. *Metallomics*, **4**:78-90.

Hongna Sun1, Yanmei Yang1, Hanwen Shao1, Weiwei Sun1, Muyu Gu1, Hui Wang1,Lixin Jiang1, Lisha Qu1, Dianjun Sun1 and Yanhui Gao. (2017). Sodium Arsenite-Induced Learning and Memory Impairment Is Associated with Endoplasmic Reticulum Stress-Mediated Apoptosis in Rat Hippocampus.

Huo, T.-G., Li, W.-K., Zhang, Y.-H., Yuan, J., Gao, L.-Y., Yuan, Y., et al. (2015). Excitotoxicity induced by realgar in the rat hippocampus: the involvement of learning memory injury, dysfunction of glutamate metabolism and NMDA receptors. *Mol. Neurobiol.* 51: 980–994.

Inouye B, Morita K, Ishida T, Ogata M. (1980). Cooperative effect of sulfite and vanadium compounds on lipid peroxidation. Toxicol Appl Pharmacol 53:101-107.

Irfan Zia Qureshi, Asia Bibi, Sana Shahid, Madiha Ghazanfar.(2016). Exposure to sub-acute doses of fipronil and buprofezin in combination or alone induces biochemical, hematological, histopathological and genotoxic damage in common carp (*Cyprinus carpio* 197: 103-114.

Iwa Leea, Per Eriksson, Anders Fredriksson, Sonja Buratovic, Henrik Viberg. (2015). Developmental neurotoxic effects of two pesticides: Behavior and neuroprotein studies on endosulfan and cypermethrin. Toxicology 335: 1–10.

J Genet Boyd, E. M. (1959). The acute oral toxicity of acetylsalicylic acid. Toxicol Appl Psychol 56, 439–53.

Jaijoy K, Soonthornchareonnon N, Lertprasertsuke N, Panthong A, Sireeratawong S. (2010). Acute and chronic oral toxicity of standardized water extract from the fruit of *Phyllanthus emblica*Linn. Int. J Appl Res Natl Prod; 3:48–58

Jullyana de Souza Siqueira Quintans , Paula Passos Menezes , Márcio Roberto Viana Santos ,Leonardo Rigoldi Bonjardim , Jackson Roberto Guedes Silva Almeida , Daniel Pens Gelain ,Adriano Antunes de Souza Araújo , Lucindo José Quintans-Júnior .(2013). Improvement of p-cymene anti nociceptive and anti-inflammatory effects by inclusion in – cyclodextrin. Phytomedicine 20: 436–440.

jürg Zarn1 and Maria Tasheva.(2008). Nutritional and Toxicological Risks Section, Swiss Federal Office of Public Health, Zurich, Switzerland; and National Service for Plant Protection, Ministry of Agriculture and Food, Sofia, Bulgaria. *BUPROFEZIN 35–80 JMPR 2008.*

Kai Chen Xiao-Mei Liu, Rong Li, Yuan Liu, Hai Hu, Shun-Peng Li, Jian-Dong Jiang. (2011). Isolation of a buprofezin co-metabolizing strain of *Pseudomonas* sp. DFS35-4 and identification of the buprofezin transformation pathway.22: 1135–1142.

Kasi Marimuthu Narmataa Muthu, Rathinam Xavier, Jesu Arockiaraj, M. Aminur Rahman, Sreeramanan Subramaniam. (2013). Toxicity of buprofezin on the Survival of Embryo and Larvae of African Catfish, *Clarias gariepinus* (Bloch). PLoS ONE 8(10): e75545.

Ku, T., Yan, W., Jia, W., Yun, Y., Zhu, N., Li, G., Sang, N. (2015). Characterization of synergistic embryotoxicity of nickel and buprofezin in zebrafish. Environ. Sci.Technol. 49: 4600–4608.

Küçükatay V, Genç O, Kocamaz E, Emmungil G, Erken H, Bagci H. (2008) Spinal reflexes in normal and sulfite oxidase deficient rats: effect of sulfite exposure. Toxicol Ind Health 24:147-153

Küçükatay V, Savcioğlu F, Hacioğlu G, Yargiçoğlu P, Ağar A.(2005). Effect of sulfte on cognitive function in normal and sulfite oxidase deficient rats. Neurotoxicol Teratol 27:47-54.

Kuhad A, Chopra K. (2007). Curcumin attenuates diabetic encephalopathy in rats: behavioral and biochemical evidences. Eur J Pharmacol 576:34-42

L. Mathew, A. Vale, J.E. Adcock. (2010). Arsenical peripheral neuropathy, Pract. Neurol. 10: 34–38

Landrigan, P. J., Claudio, L., Markowitz, S. B., Berkowitz, G. S., Brenner, B. L. Romero, H., et al. (1999). Pesticides and inner-city children: exposures, risks,and prevention. *Environ. Health Perspect.* 107:431–437.

Lin MS, Lee YH, Chiu WT, Hung KS. (2011). Curcumin provides neuroprotection after spinal cord injury. J Surg Res166:280-289

Lola Roldan-Tapia Francisco A.Nieto-Escamez Eva M.del Aguila Francisco Laynez Tesifon Parron Fernando Sanchez-Santed.(2006). Neuropsychological sequelae from acute poisoning and long-term exposure to carbamate and organophosphate pesticides. Neurotoxicology and Teratology 28:694-703.

Lu, G. L., Chang, Y. C., Chen, Y. H., Tsai, C. L., and Tsai, M. C. (2009). Arsenic trioxide modulates the central snail neuron action potential. *J. Formos. Med.Assoc.* 108, 683–693.

Lucio G. Costa1, 2, Gennaro Giordano1, Marina Guizzetti1, Annabella Vitalone. (2008). Neurotoxicity of pesticides: a brief review .Frontiers in Bioscience 13:1240-1249

Ma WP, Cao J, Tian M, Cui MH, Han HL, Yang YX, et al. (2007). Exposure to chronic constant light impairs spatial memory and influences long-term depression in rats. Neurosci Res.59:224–30

Mancinelli, A., Borsini, F., D'Aranno, V., Lecci, A., Meli, A. (1988). Cholinergic drug effects on antidepressant-induced behaviour in the forced swimming test. Eur. J. Pharmacol.158, 199–205

Mao, J., Yang, J., Zhang, Y., Li, T., Wang, C., Xu, L., et al. (2016). Arsenic trioxide mediates HAPI microglia inflammatory response and subsequent neuron apoptosis through p38/JNK MAPK/STAT3 pathway. *Toxicol. Appl. Pharmacol.* 303, 79–89.

Marimuthu, K., Muthu, N., Xavier, R., Arockiaraj, J., Rahman, M.A., S. (2013). Toxicity of buprofezin on the survival of embryo and larvae of african catfish, clarias gariepinus (Bloch). PLoS One 8: 4–9.

Mark E. Nevins Susan A. Nash Patrick M. Beardsley.(1993). Quantitative grip strength assessment as a means of evaluating muscle relaxation in mice. *Psychopharmacology* 110: 92–96

Martin K Johnson, Dag Jacobsen, Tim J Meredith, Peter Eyer, Andrew J Heath,David A Ligtenstein, Tim C Marrs, Ladislaus Szinicz, J Allister Vale and John A Haines.(2000). Evaluation of antidotes for poisoning by organophosphorus pesticides. *Emergency Medicine* **12**: 22–37.

Martin SJ, Clark RE (2007) .The rodent hippocampus and spatial memory: from synapses to systems. Cell Mol Life Sci 64: 401–431.

Mattfeld, A. T., and Stark, C. E. (2015). Functional contributions and interactions between the human hippocampus and subregions of the striatum during arbitrary associative learning and memory. *Hippocampus* 25:900–911.

Mehdi Goudarzi, Shivasadat Amiri, Ali Nesari, Azam Hosseinzadeh, Esrafil Mansouri, Saeed Mehrzadi.(2018). The possible neuroprotective effect of ellagic acid on sodium arsenate induced neurotoxicity in rats. Life sciences 198: 38-45.

Mehdi Goudarzi, Shivasadat Amiri, Ali Nesari, Azam Hosseinzadeh, Esrafil Mansouri, Saeed Mehrzadi.(2018). The possible neuroprotective effect of ellagic acid on sodium arsenate induced neurotoxicity in rats. Life sciences 198: 38-45

Meng Z, Sang N, Zhang B. (2002). Effects of derivatives of sulfur dioxide on micronuclei formation in mouse bone marrow cells in vivo. Bull Environ Contam Toxicol 69:257-264.

Mohamed E. I. Badawy,Anter Kenawy, and Ahmed F. El-Aswad.(2013). Toxicity Assessment of buprofezin, Lufenuron,

andTriflumuron to the Earthworm *Aporrectodea caliginosa* International Journal of Zoology Volume 2013, Article ID 174523, 9

Morris RGM, Garrud P, Rawlins JNP, O'Keefe J. (1982). Place navigation impaired in rats with hippocampal lesions. Nature.297:681–3

Morris RGM. (1981). spatial localization does not depend on the presence of localcues.LearnMotiv.12:239–60.

Mudd SH, Irreverre F, Laster L. (1967). Sulfite oxidase deficiency in man: demonstration of the enzymatic defect. Science 156:1599-1602

Muralidhara S, Ramanathan R, Mehta SM, Lash LH, Acosta D, Bruckner JV.(2001). Acute, subacute, and subchronic oral toxicity studies of 1,1-dichloroethane in rats: Application to risk evaluation. Toxicological screening **2**(2): 74–79.

Noorafshan A, Ashkani-Esfahani S. (2013).A review of therapeutic effects of curcumin. Curr Pharm Des 19:2032-2046.

Opinion on Genotoxic and Carcinogenic Potential of buprofezin in the Context of the Human Risk Assessment. (2007).

Page ME, Lucki I. (2002). Effects of acute and chronic reboxetine treatment on stress-induced monoamine efflux in the rat frontal cortex Neuropsychopharmacology. 27(2):237–47.

Parasuraman, S. (2011) .Toxicological screening. Journal of pharmacology and pharmacotherapeutics, 2 (2):74

Patil Satyajit G, Amol R. Padol and Balakrishnamurthy P. (2015). Evaluation of neurobehavioral effects of acrylamide under functional observational battery (fob) in rats on repeated oral exposure. patil satyajit g et al. / journal of pharmaceutical biology, 5(1): 22-28

Peter A. Hancock Deborah R. Billings, Kristin E. Schaefer, Jessie Y. C. Chen, Ewart J. de Visser, Raja Parasuraman.(2011). A Meta-Analysis

Yoshio Izawa, Matazaemon Uchida, Tatsuyoshi Sugimoto, Toshiro Asai., (1985). Inhibition of chitin biosynthesis by buprofezin analogs in relation to their activity controlling *Nilaparvata lugens* Stål. *Pesticide Biochemistry and Physiology* 24:343-347

Zen Huat Lu, Charles T. Dameron, Marc Solioz. (2003). The *Enterococcus hirae* paradigm of copper homeostasis: Copper chaperone turnover, interactions, and transactions. 16: 137–143.