STUDY ON EVALUATION OF THERAPEUTIC EFFECT OF *BACOPA-MONNIERI* ON CEREBELLAR TOXICITY CAUSED BY ARSENIC AND NICOTINE IN MALE SPRAGUE-DAWLEY RATS

THESIS SUBMITTED

TO

SRI DEVARAJ URS ACADEMY OF

HIGHER EDUCATION AND RESEARCH

For Awarding the Degree as

DOCTOR OF PHILOSOPHY

IN MEDICAL ANATOMY

Under Faculty of Medicine

By

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Under the Supervision of

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I 'Ravi Shankar Prasad Sawan' do hereby declare that the thesis entitled **"Study on evaluation of therapeutic effect of *Bacopa-Monnieri* on Cerebellar toxicity caused by Arsenic and Nicotine in Male Sprague-Dawley Rats"** embodies original research work carried out by me for the award of **Doctor of Philosophy** in Medical Anatomy under Supervision of **Dr. Sridevi NS**, Professor and Head, Department of Anatomy and Co-supervision of **Dr. Shashidhar KN,** Professor and Head, Department of Biochemistry, Sri Devaraj Urs Medical College, A Constituent of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India.

This work has not been submitted in part or full for any other degree or diploma of this or any other Universities. The particulars given in this thesis are true to the best of my knowledge.

Date: 27-10-2022

Place: SDUMC, KOLAR

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(Ravi Shankar Prasad Sawan)

CERTIFICATE

This is to certify that the thesis entitled **"Study on evaluation of therapeutic effect of *Bacopa-Monnieri* on Cerebellar toxicity caused by Arsenic and Nicotine in Male Sprague-Dawley Rats"** submitted for the requirement of award of Degree, Doctor of Philosophy by **Mr. Ravi Shankar Prasad Sawan (Reg. No. 18PY1002)** is the record of original research work carried out by him under supervision of **Dr. Sridevi NS,** Professor and Head, Department of Anatomy and Co-Supervision of **Dr. Shashidhar KN,** Professor and Head, Department of Biochemistry, Sri Devaraj Urs Medical College, A constituents of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka, India.

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Authentication Certificate

This is to certify that the plant specimen brought for identification collected by Ravi Shankar Prasad Sawan is identified as *Bacopa monnieri* (L.) Wettst. (Plantaginaceae) and given the accession number **UASB4608**.

To,

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At this juncture, I come to the notion that this work is the result of wholehearted cooperation of the well-wishers and grace of Almighty God.

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Ravi Shankar Prasad Sawan

LIST OF ABBREVATIONS

CNS	Central Nervous System
PNS	Peripheral Nervous System
As	Arsenic
Ni	Nicotine
BM	*Bacopa-Monnieri*
WHO	World Health Organization
BBB	Blood Brain Barrier
MCL	Maximum contaminant limit
PCs	Purkinje cells
ROS	Reactive Oxygen Species
iAs	Inorganic Arsenic
nAChRs	Nicotinic acetylcholine receptor
IUCN	International Union for Conservation of Nature
As_2O_3	Arsenic trioxide
PPM	Parts per million
$NaAsO_2$	Sodium Arsenite
$Ascl_3$	Arsenic trichloride
As_2O_5	Arsenic pentoxide
$PbHAsO_4$	lead arsenate
OFT	Open Field Test
EPM	Elevated Plus Maze
BWT	Beam Walking Test
H & E	Hematoxylin and Eosin
DPX	Dibutylphthalate polystyrene xylene
NRT	Nicotine replacement therapy
HP	Hydrogen Peroxide
MeHg	Methylmercury
LPP	Lipid peroxidation product
BME	Extract of *Bacopa-Monnieri*
IAEC	Institutional Animal Ethics Committee
PBS	Phosphate Buffer solution

CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
HPLC	High Performance Liquid Chromatography
AAS	Atomic Absorption Spectrophotometry
MDA	Malondialdehyde
NO	Nitric-Oxide
GPx	Glutathione Peroxidase
LPO	Lipid Peroxidation
HNO_3	Nitric-acid
GSH	Reduced Glutathione
EDTA	Ethylene diamine tetra-acetic acid
$HClO_4$	Perchloric acid
TCA	Trichloro-Acetic Acid
TBARS	Thiobarbituric Acid Reactive Substances
µl	Microliter
µg	Microgram
%	Percentage
SD	Standard Deviation
RPM	Revolutions per minute
µm	Micrometer/micron

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Introduction

"We are what our nervous system permits" that means our thoughts, feelings, emotions, sensations, desires, dreams, ideas, creative urges, language and life itself are under the control of the most complex structure in the world – T**he Nervous System.** The functions of more than 100 billion neurons constituting the nervous system are one of the mysteries of science. The nervous system is a complex collection of nerves and specialized cells called as neurons which transmit the signals between different parts of the body [1]. Sensory nerves gather information from the environment; send that information to spinal cord, which then sends a speed message to the brain. Motor neurons deliver the instructions from the brain to the rest of our body and adjust the body to the surroundings and regulate all bodily activities both voluntary and involuntary [2].

The Nervous system is commonly partitioned in two parts: the **Peripheral Nervous System (PNS)** and the **Central Nervous System (CNS)**. PNS merges into the CNS. So, dividing the Nervous system in two separate parts but in actual, these two interacting and communicating systems are a continuous entity. Together, the CNS and PNS control every part of our daily life, from breathing and blinking to learning and remembering, the way we walk and talk and it also control the things that we are less aware of - like beating of heart and digestion of food [2].

Furthermore, The CNS is usually subdivided into **Brain** (lies in cranial cavity) and **Spinal Cord** (lies in upper $2/3^{rd}$ of the vertebral canal). The brain is again subdivided into three main parts:

1. **Prosencephalon (Forebrain)**
    a) Telencephalon: Cerebrum
    b) Diencephalon: Thalamus, hypothalamus, metathalamus, subthalamus and epithalamus

2. **Mesencephalon (Midbrain):** Crus cerebri, Substantia nigra, tectum and tegmentum
3. **Rhombencephalon (Hindbrain)**
    a) Metencephalon: Pons and cerebellum
    b) Myelencephalon: Medulla oblongata

The cerebellum (Latin word means little brain) is a part of hindbrain situated in posterior cranial fossa behind the pons, medulla oblongata and fourth ventricle and separated from occipital lobe of cerebrum by tentorium cerebelli. It is somewhat ovoid in shape. It weighs about 150 grams in male, forms $1/10^{th}$ part of cerebrum in adult and $1/20^{th}$ part in infants [3]. Cerebellum develops from the dorsolateral part of alar lamina of the metencephalon [4]. It helps in regulation and co-ordination in movement, posture and balance [5]. However, numbers of anatomical, physiological, clinical and neuro-imaging studies have shown that the cerebellum has extensive connections with the cerebral networks which may also play a role in neuro-cognition [6-9].

Cerebellar dysfunction causes ataxia, dysmetria, dyssynergia and disdiadikinesis of voluntary movement, disorders of gait and equilibrium, disorders of speech and abnormal ocular movements [10,11]. Diseases involving the cerebellum occur relatively common in children and adults around the globe. The prevalence rate of cerebellar ataxia is 26/100,000 in children, a prevalence rate of dominant hereditary cerebellar ataxia of 2.7/100,000 and a prevalence rate of recessive hereditary cerebellar ataxia of 3.3/100,000 [12].

CEREBELLAR TOXICITY:

There are a wide variety of causes for cerebellum neurotoxicity and leads to cerebellar ataxia **[13, 14, 15].**

1. **Nutritional deficiencies**
    - **a)** Thiamine deficiency
    - **b)** Vitamin B12 deficiency
    - **c)** Vitamin E deficiency
    - **d)** Zinc deficiency (rarely).
2. **Infections**
    - **a) Acute cerebellitis** (Epstein-Barr virus, influenza A and B, mumps, varicella-zoster, coxsackie virus, rotavirus, echovirus, Mycoplasma and immunization
    - **b) Bacterial:** Meningo-encephalitis or intracranial abscess, Mycoplasma pneumoniae, Listeria monocytogenes
    - **c) Viral:** acute infections (eg, varicella); chronic infections - eg, human immunodeficiency virus (HIV); post-viral syndromes (eg, post-infective cerebellar syndrome in childhood)
    - **d) Parasitic:** (eg. toxoplasma, falciparum malaria, Lyme disease)
    - **e) Other infectious** (Lyme disease, Whipple disease, Aspergillus, JC virus, syphilis and Creutzfeldt-Jakob disease)
3. **Toxins**:
    - a) Alcohol
    - b) Environmental Heavy metals (mercury, lead, **Arsenic**, manganese, aluminium, thallium, germanium, uranium, vanadium)
    - c) Solvents

d) carbon-monoxide poisoning

4. **Structural and vascular causes:**

    a) Cerebellar stroke (ischemic and hemorrhagic)

    b) Tumors (primary and metastasis), Abscesses

5. **Drugs**:

    a) Anticonvulsants drugs (phenytoin, carbamazepine etc.)

    b) Antineoplastic drugs (methotrexate, capecitabine, epothilone D) and

    c) Other drugs (lithium salts, amiodarone, procainamide, bismuth, mefloquine, cimetidine, metronidazole, **Nicotine**)

6. **Drug abuse and addiction**:

    a) Cocaine

    b) Heroin

    c) Phencyclidine

    d) Methadone

7. **Drug overdose**:

    a) Accidental temazepam overdose in children

8. Trauma

9. Multiple sclerosis

10. Congenital

11. Paraneoplastic cerebellar degeneration

12. Insecticides/herbicides

ARSENIC:

In 21st century world, increasing industrialization of societies, expansion of factories, agricultural activities and burning of fossil fuels has dramatically increased

the amount of pollutants and environmental pollution. Environmental pollution is the world's greatest problem facing humanity and the leading environmental causes of morbidity and mortality. In 2015, it was reported that ill-health caused by environmental pollution accounted for 9 million premature deaths, which is more than three times the number of deaths from malaria, AIDS and tuberculosis put together [16].

Of all the types of pollution, contamination due to heavy metals and metalloids such as lead (Pb), cadmium (Cd), methylmercury (MeHg) and arsenic (As) are wide spread [17]. Among these heavy metals, environmental pollution due to arsenic is a major toxin and has become a major public health challenge in many countries such as Bangladesh, India, Nepal, Taiwan, Mongolia, Vietnam, Pakistan, China, Afghanistan, Argentina and USA [18]. Arsenic is released to the environment from anthropogenic sources such as mining wastes, metal smelting, wood preservatives, glass and semiconductor manufacture, pesticides and herbicides production [19]. Due to its increasing production and utilization, arsenic is ubiquitously present in water, air and soil affecting occupational workers as well as general population [20]. For the general population, arsenic in drinking water is the main exposure source and more harmful than arsenic in food because the bioavailability (actual amount absorbed into the bloodstream) of arsenic from water is greater than that from grains or vegetables [21]. Arsenic in drinking water is mainly inorganic arsenic which is more toxic than organic arsenic.

Arsenic primarily enters into the body through direct consumption of drinking water from geological deposits by drilling of tube well [22]. WHO and other regulatory bodies have established a maximum contaminant limit (MCL) of 10µg/L inorganic arsenic (iAs) in drinking water for the safety of human health based on

reducing cancer risk, but this limit is not considered as an endpoints for other cancers [23]. Globally, more than 200 million people are affected by ground water contaminated with arsenic concentration greater than 10µg/L [24]. During recent years, in comparison to others, China and India have become the most affected countries with serious diseases and the largest number of victims seen in hospitals due to arsenic poisoning [25]. In India, 7 states are reported to be affected by ground water arsenic contamination, and approximately 30 million individuals are consuming arsenic laden drinking water [26].

Numerous experimental and epidemiological studies provide evidence that acute and chronic arsenic exposure has been linked with numerous chronic indices in all human and animal organ systems and contributes to a wide spectrum of diseases especially disease involving CNS [27,28,29].

Arsenic crosses the Blood Brain Barrier (BBB) and accumulates in different regions of the brain and causes neurotoxicity [30]. The neurotoxicity of arsenic is widely noticed since arsenic exposure have shown various neurological side effects such as vertigo, sleep disorder, impaired coordination of movements, uncontrolled motor learning, loss of skilled voluntary movements, impaired learning, memory and concentration [31,32]. Brain is the first target organ for arsenic toxicity causing oxidative stress which increases the release of free radicals in brain leading to apoptosis of neural cells [18]. Arsenic induced toxicity causes morphological, structural and pathological changes in the brain [33]. Reports in the literature also document that arsenic treated rats reduces neuronal viability in primary cultures of rat cerebellar neurons and showed alterations in locomotor behavior and learning task [34,35]. These literatures indicate that cerebellum can be affected by arsenic.

NICOTINE:

Tobacco use is world's most serious problem, with substantial effect on human health and economic consequences. Tobacco exposure in any form (by inhalation of second hand tobacco smoker or comes in contact with the spit of oral smokeless tobacco users or with tobacco leaves during farming or manufacturing) kills and sickens millions of people every year [36]. Every year, Smoking related diseases causes more death than any other diseases (such as HIV, illegal drugs and alcohol use, motor vehicle accidents and murders combined) [37,38]. It is also a major contributor to morbidity, reducing fertility and increased risk of most cancers, coronary heart disease, diabetes and countless health complications [39].

Tobacco and tobacco smoke contains more than 9000 chemicals. Many of which are toxic and carcinogenic [40]. The tobacco alkaloid 'Nicotine' is considered as the major addictive component and toxic alkaloid compound in tobacco smoke and found primarily in the roots and leaves of solanoceous plant family where it constitutes approximately 0.60-3.00% of dry weight of tobacco [41,42]. There are different routes to get exposed with nicotine including cigarette smoking, chewing tobacco, holding moist snuff in the mouth, inhaling dry snuff through the nose, inhaling smoke from a water pipe and inhaling vapor from an electronic cigarette [43,44,45,46].

Nicotine exhibits protective activity towards several diseases such as Alzheimer's and Parkinson's diseases [47]. It was also used to treat nicotine dependence to eliminate smoking or minimize addiction and the damage it does to the health by providing controlled level of nicotine to the patient through gums, dermal patches, lozenges, inhalers or nasal sprays [48]. However, with extensive consumption, nicotine produces various detrimental roles on different systems of the

body such as cardiovascular system, respiratory system, gastrointestinal system, reproductive system, especially the Nervous System and triggers pathophysiology of brain and causes neurodegeneration. Exposure of the experimental animals to nicotine elicits oxidative stress and histological changes due to impairment in the brain's structural integrity and function [49, 50, 51].

By cigarette smoke or tobacco chewing, nicotine enters into the body through absorption in mucosal lining of mouth, nose and alveoli of lung. From the lungs, nicotine mixes into the bloodstream and rapidly enters into the brain by crossing Blood Brain Barrier (BBB) within 10-20 seconds, faster than intravenous administration and gets concentrated in the brain showing alterations in multiple brain regions such as thalamus, prefrontal cortex and cerebellum [52,53]. Within the brain, nicotine binds with nicotinic acetylcholine receptor (nAChRs) and disturbs cholinergic system, neuronal migration, synaptogenesis and neurotransmitter release. These nicotine induced adverse effects can alter the brain activity and produce neurobehavioral impairment such as cognitive effects, an influence of anxiety, analgesia or depression like behavior [54,55].

Nicotine causes significant loss of white matter and reduction in number of Purkinje cells of cerebellum, with possible predisposition to progressive impairment in the structural integrity and function of the cerebellum [56,57]. Though, nicotine causes postural imbalance, showing that nicotine might affect the circuitry involving the cerebellum in smokers [58]. Therefore, chronic exposure to nicotine negatively impacts the cerebellum, leading to impaired behavior and cognitive function.

The excessive production of free radicals and Reactive oxygen species (ROS) plays an important role in nicotine-induced brain (structural or neurobehavioral) alterations. The increased production of ROS and free radicals by nicotine can

produce a condition of oxidative stress which may cause cell death in various regions of the brain and memory impairments [59, 60]. These documents showed that cerebellum can be affected by nicotine.

BACOPA MONNIERI:

Medicinal plants are a pivotal reservoir of pharmacologically active compounds and have been used as a therapeutic medicine for several diseases since the ancient period. Recently, the interest in the use of medicinal plants has grown dramatically all around the world and almost 80,000 flowering plants are used for pharmaceutical purposes as per the International Union for Conservation of Nature (IUCN) report [61]. The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspects of primary health care [62].

In Indian traditional medicine, *Bacopa-Monnieri (BM)* is an important medicinal plant used in Ayurveda, a holistic system of medicine originating from India. It is a small perennial herbaceous plant commonly known as 'Brahmi' belonging to the family Scrophulariaceae [63]. *BM* is widely distributed in warmer and wetland region of the world, apart from India, Nepal, Vietnam, Sri Lanka and also found in Florida and other Southern states of USA [64]. It has been claimed as a nerve tonic and extremely used for the treatment of various neurological conditions (such as antidepressant, anxiolytic, anticonvulsant and antiparkinsonian) and also studies have shown to improve learning, memory and concentration [65, 66, 67]. It is also used in the repair of damaged neurons, neurological synthesis and restoration of synaptic activity and improves brain function [61].

Lacunae of the study:

There are numerous reports stating that arsenic or nicotine individually may lead to various health problems affecting different systems like reproductive, cardiovascular, respiratory, renal, digestive and nervous etc. The previous published reports also indicate that arsenic or nicotine individually can also impair the neurobehavioral activities such as cognition, learning and memory.

However, neurobehavioral, neurochemical and histomorphological changes in cerebellum due to arsenic or nicotine individually or in combination are not conclusive.

The previous studies have not established protective agent that reverses the alterations caused by arsenic or nicotine individually or in combination. In this view, an important Indian traditional medicinal plant; *Bacopa-Monnieiri* was used to assess its amelioration against the alterations caused by Arsenic or Nicotine individually or in combination.

Therefore, the present study was carried out in "ameliorative effect of *Bacopa-Monnieri* on cerebellar toxicity caused by arsenic or nicotine individually or in combination of arsenic and nicotine in male Sprague-Dawley Rats."

Objectives

RESEARCH QUESTIONS

1) Do Arsenic and Nicotine individually or in combination affect the Behavior, biochemical and Motor activities associated with Histomorphology of Cerebellum in Male Sprague-Dawley Rats?
2) Does *Bacopa-Monnieri* ameliorate the Arsenic and Nicotine individually or in combination induced Behavioral, biochemical and Motor activities associated with the Histomorphology of Cerebellum in Sprague-Dawley Rats?

AIM

To find the effect of Arsenic and Nicotine individually or in combination on the Biochemical, Behavioral, Motor and Histomorphology of cerebellum and the impact of *Bacopa-Monnieri* on these activities.

OBJECTIVES:

1) To assess the effect of Arsenic and Nicotine individually or in combination on Behavioral and Motor activities in male Sprague-Dawley rats
2) To estimate the biochemical changes in serum and cerebellum of Arsenic and Nicotine individually or in combined exposed rats
3) To investigate the Histomorphological effect of Arsenic, Nicotine and Co-exposure on rat cerebellum
4) To assess the concentration of arsenic and nicotine in Arsenic, Nicotine and Co-exposure induced rats
5) To determine the ameliorative effect of *Bacopa-Monnieri* on Arsenic, Nicotine and Co-exposure induced alterations in:-
    a) Behavior and Motor activities
    b) Oxidative stress markers
    c) Histomorphological changes in cerebellum

Annexure-I

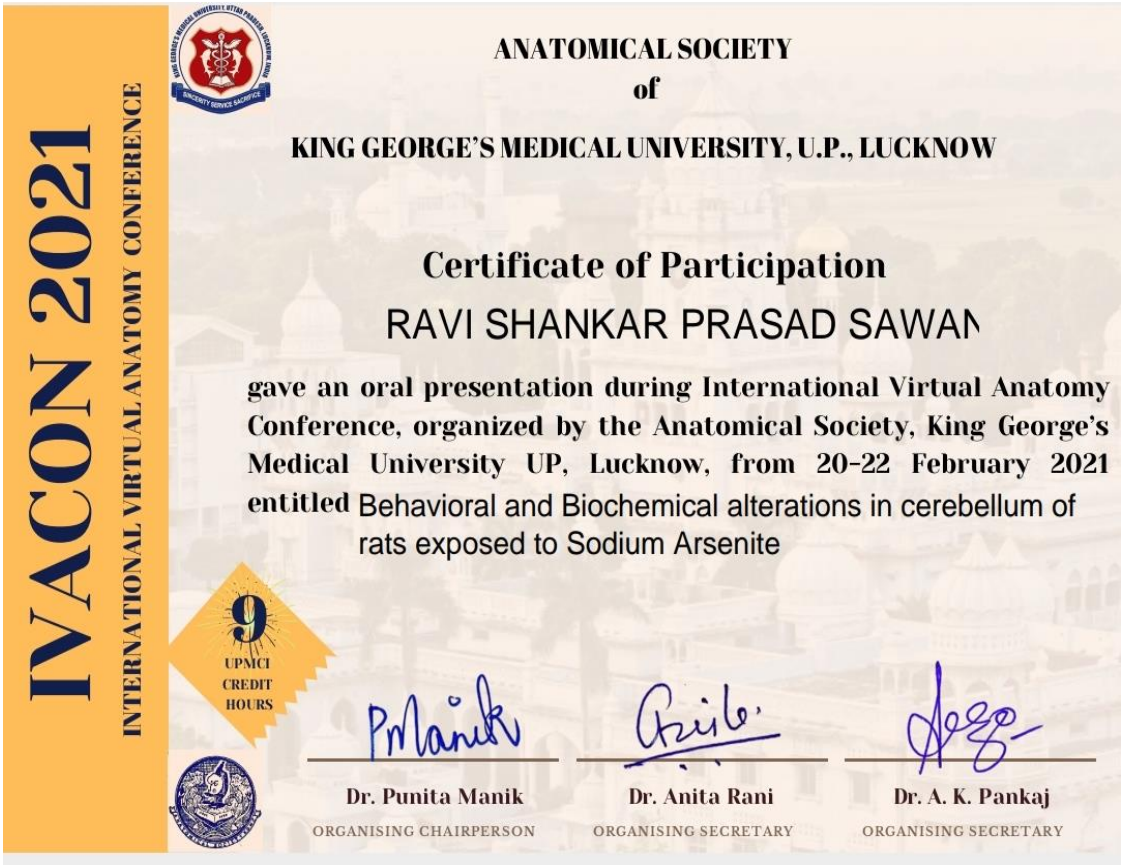
PUBLICATIONS

Paper Title	Authors	Journal	Indexation
Ameliorating Effect of Bacopa-Monnieri against Nicotine Induced Cerebellar Toxicity in Male Sprague-Dawley rats	**Ravi Shankar Prasad Sawan[1]** Sridevi NS[2] Shashidhar K.N[3]	Biomedical and Pharmacology Journal (BPJ). 2022; 15(2)	Scopus
Neurobehavioural and Neurochemical Changes in Arsenic Induced Cerebellar Toxicity in Male Sprague-Dawley Rats: An Experimental Study.	**Ravi Shankar Prasad Sawan[1]** Sridevi NS[2] Shashidhar K.N[3]	Journal of Clinical and Diagnostic Research. 2022; 16(3)	Web of Science

PRESENTATIONS

Title	Authors	Conference/ Webinar	Date
Behavioral and biochemical alterations in cerebellum of rats exposed to sodium Arsenite **(Oral)**	**Ravi Shankar Prasad Sawan[1]** Sridevi NS[2] Shashidhar K.N[3]	International conference-IVACON	20-22nd Feb. 2022
Neurobehavioral and neurochemical changes in nicotine induced rat cerebellum **(Oral)**	**Ravi Shankar Prasad Sawan[1]** Sridevi NS[2] Shashidhar K.N[3]	National conference-AVEOCON	9-10th July 2021

Annexure-II



IVACON 2021

INTERNATIONAL VIRTUAL ANATOMY CONFERENCE

ANATOMICAL SOCIETY
of
KING GEORGE'S MEDICAL UNIVERSITY, U.P., LUCKNOW

Certificate of Participation

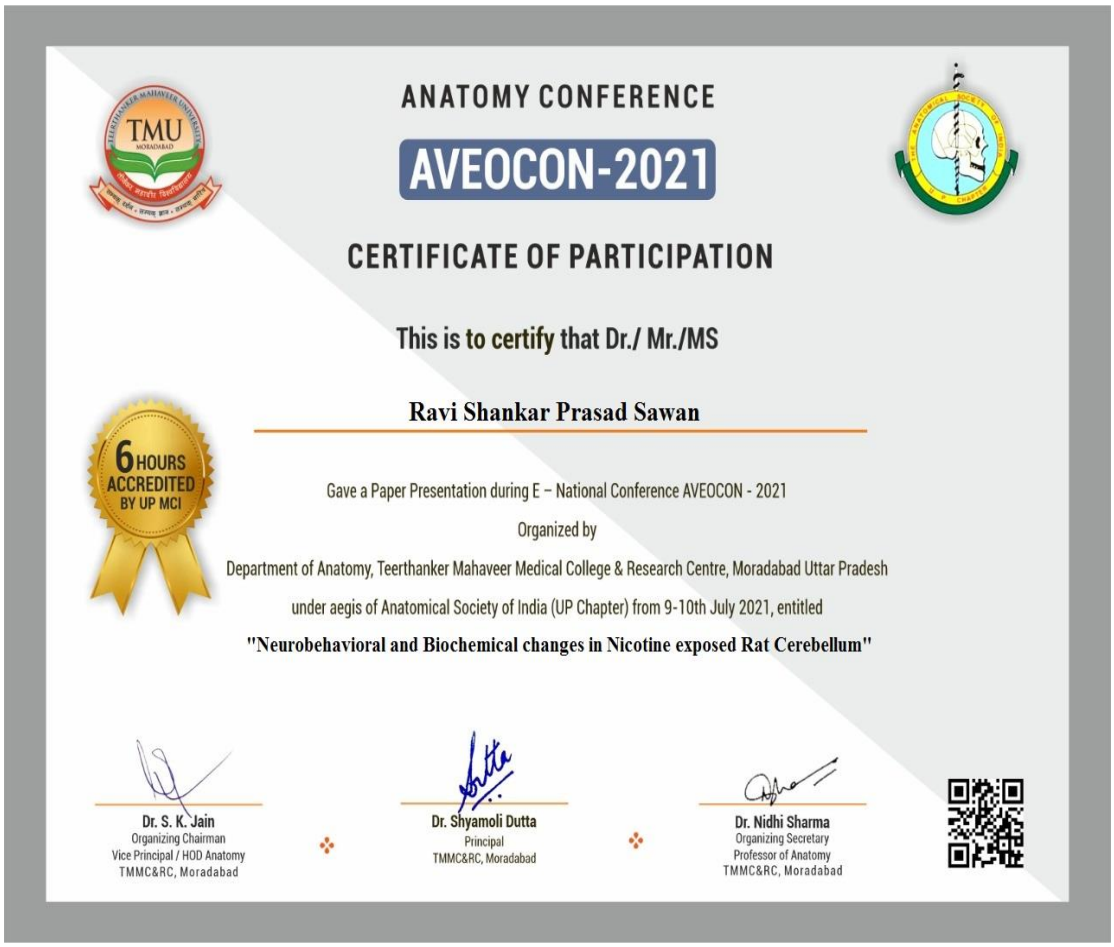
RAVI SHANKAR PRASAD SAWAN

gave an oral presentation during International Virtual Anatomy Conference, organized by the Anatomical Society, King George's Medical University UP, Lucknow, from 20-22 February 2021 entitled Behavioral and Biochemical alterations in cerebellum of rats exposed to Sodium Arsenite

9 UPMCI CREDIT HOURS

Dr. Punita Manik	Dr. Anita Rani	Dr. A. K. Pankaj
ORGANISING CHAIRPERSON	ORGANISING SECRETARY	ORGANISING SECRETARY

Annexure-III



ANATOMY CONFERENCE

AVEOCON-2021

CERTIFICATE OF PARTICIPATION

This is to certify that Dr./ Mr./MS

Ravi Shankar Prasad Sawan

6 HOURS ACCREDITED BY UP MCI

Gave a Paper Presentation during E – National Conference AVEOCON - 2021

Organized by

Department of Anatomy, Teerthanker Mahaveer Medical College & Research Centre, Moradabad Uttar Pradesh

under aegis of Anatomical Society of India (UP Chapter) from 9-10th July 2021, entitled

"Neurobehavioral and Biochemical changes in Nicotine exposed Rat Cerebellum"

Dr. S. K. Jain
Organizing Chairman
Vice Principal / HOD Anatomy
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Dr. Shyamoli Dutta
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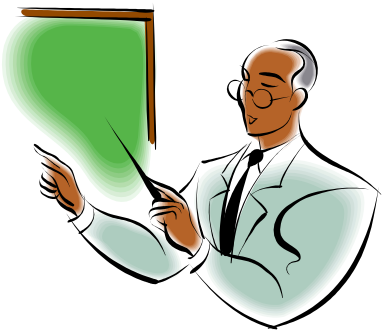
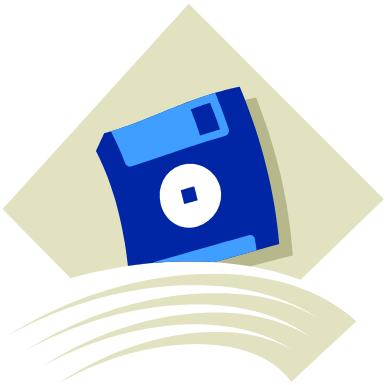


Conclusion

1. Results of the present study suggest that arsenic and nicotine individually or in combination of arsenic and nicotine could be considered as a cause of cerebellar toxicity in male Sprague-Dawley rats due to:
    a) Increased oxidative stress in cerebellum
    b) Reduction in locomotion
    c) Increased anxiety
    d) Loss of motor coordination and balance
    e) Reduction in number of purkinje cells and shrinkage of purkinje cells
    f) Alteration in thickness of molecular layer and granular layer of cerebellum
    g) Reduction in cortical thickness at folium, fissure and base
2. Results of the present study also demonstrated that combined exposure of arsenic and nicotine reduced the body weight and brain weight.
3. Further, the present study may also suggest that *Bacopa-Monnieri* could be considered as a therapeutic agent against arsenic and /or nicotine treated male Sprague-Dawley rats due to:
    a) Reduced oxidative stress
    b) Improvement in locomotion
    c) Reduced anxiety
    d) Improvement in motor coordination and balance
    e) Improvement in number of purkinje cells and cell shrinkage of Purkinje cells
4. The oral supplementation of Bacopa-Monnieri brought the body weight and brain weight to normal level which was altered due to arsenic and/or nicotine treated rats.

As shown the results, the present study conclude that arsenic and nicotine individually or a combination of arsenic and nicotine exposure followed by Bacopa-Monnieri treated rats indicated the protective influence of Bacopa-Monnieri in cerebellum of rats against arsenic and nicotine alone or a combination of arsenic and nicotine treated rats.

- Only selected markers and behavioral tests were evaluated
- Quantitative phytochemical analysis of *Bacopa-Monnieri* was not done
- Sample size is small in each group.



New Knowledge Generated

1. Arsenic, nicotine and a combination of arsenic and nicotine treated rats reduced the body weight gain. Whereas Arsenic and nicotine individually and a combination of arsenic and nicotine exposure followed by *Bacopa-Monnieri* treated rats reduces the body weight loss caused by arsenic and nicotine individually or in combination of arsenic and nicotine treated rats.
2. Arsenic and/or nicotine significantly increases the oxidative stress in serum and cerebellum of rats. *Bacopa-Monnieri* treated rats significantly reduces the oxidative stress in serum and cerebellum of rats.
3. Arsenic and nicotine individually or a combination of arsenic and nicotine treated rats showed anxiety like behaviors and alterations in motor coordination and balance as well as reduction in locomotion activities. Whereas, oral supplementation of *Bacopa-Monnieri* treated rats showed improvement in anxiety like behaviors, motor coordination and balance as well as increase in locomotion activities.
4. Reduction in number of purkinje cells, cell shrinkage as well as reduction in thickness of molecular layer and granular layer and also cortical thickness at folium, fissure and base was found in arsenic and nicotine individually or a combination of arsenic and nicotine treated rats. The oral supplementation of Bacopa-Monnieri reversed the alteration caused by arsenic and nicotine individually or combination of arsenic and nicotine treated rats.

As the human populations are also exposed to arsenic and nicotine and the effect produced on the rats can be linked to human populations due to their genetic, biological and behavioral characteristics with the rats.

Hence, *Bacopa-Monnieri* can be recommended to humans who are chronically exposed to arsenic and/or nicotine which will bring reversal effect produced by arsenic and/or nicotine.