“PHARMACOLOGICAL EVALUATION OF NEUROPROTECTIVE EFFECT OF ETHONALIC EXTRACT OF ROOTS AND RHIZOMES OF NARDOSTACHYS JATAMASI IN EXPERIMENTAL ANIMAL MODEL OF TRAUMATIC BRAIN INJURY”

A Thesis Submitted to

NIRMA UNIVERSITY

in partial fulfillment for the award of the degree of

MASTER OF PHARMACY

IN

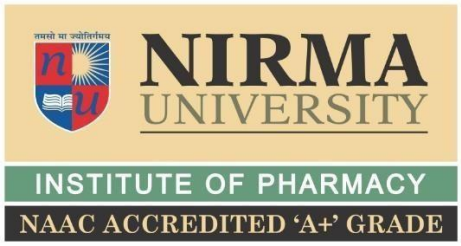
PHARMACOLOGY

BY

Misha Aanand (21MPH208), B.PHARM.

Under the guidance of

Dr. BHAGAWATI SAXENA
Assistant Professor, Department of Pharmacology
Institue of Pharmacy, Nirma University



Department of Pharmacology
Institute of Pharmacy
Nirma University
Ahmedabad-382481
Gujarat, India.

May 2023

CERTIFICATE

This is to certify that the dissertation work entitled "Pharmacological evaluation of neuroprotective effect of ethanolic extract of roots and rhizomes of Nardostachys Jatamansi in experimental animal model of traumatic brain injury" submitted by Ms. Misha Aanand (21MPH208) in partial fulfillment for the award of Master of Pharmacy in "Pharmacology" is a bonafide research work carried out by the candidate at the Department of pharmacology, Institute of Pharmacy, Nirma University under my guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

Bhagawati

Dr. Bhagawati Saxena
M.S (Pharm)., Ph.D.,
Assistant Professor,
Department of pharmacology,
Institute of Pharmacy,
Nirma University

6/10/23

Prof. Dr. Jigna Shah
M. Pharm., Ph.D.,
Head of Department,
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

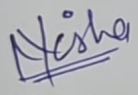
9/10/23

Prof. Dr. Gopal Natesan
M. Pharm., Ph.D.
Director
Institute of Pharmacy,
Nirma University

19th May 2023

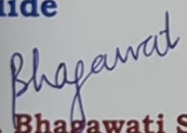
CERTIFICATE OF ORIGINALITY OF WORK

This is to undertake that the dissertation work entitled "Pharmacological evaluation of neuroprotective effect of ethanolic extract of roots and rhizomes of Nardostachys Jatamansi in experimental animal model of traumatic brain injury" Submitted by Ms. Misha Aanand (21MPH208)in partial fulfillment for the award of Master of Pharmacy in "Pharmacology" is a bonafide research work carried out by me at the "Department of Pharmacology", Institute of Pharmacy, Nirma University under the guidance of "Dr. Bhagawati Saxena". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, this work is original and not reported anywhere as per best of my Knowledge.



Ms. Misha Aanand (21MPH208)
Department of Pharmacology,
Institute of Pharmacy,
Nirma University

Guide

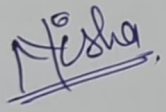


Dr. Bhagawati Saxena
M.S (Pharm)., Ph.D.,
Assistant Professor,
Department of pharmacology,
Institute of Pharmacy,
Nirma University

19th May 2023

DECLARATION

I hereby declare that the dissertation entitled "Pharmacological evaluation of neuroprotective effect of ethanolic extract of roots and rhizomes of Nardostachys Jatamansi in experimental animal model of traumatic brain injury", is based on the original work carried out by me under the guidance of Dr. Bhagwati Saxena, Assistant Professor under the Department of Pharmacology, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.



Ms. Misha Aanand (21MPH208)
Department of Pharmacology,
Institute of Pharmacy,
Nirma University,
Sarkhej - Gandhinagar Highway,
Ahmedabad-382481,
Gujarat, India

19th May 2023

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Misha Aanand

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LIST OF ABBREVIATIONS

TBI - Traumatic brain injury
NJ - Nardostachys Jatamansi
NADT - novel arm discrimination test
MDA - Malondialdehyde
NO - Nitrite Oxide
GSH - glutathione
CMC – Carboxymethyl Cellulose
NMDA - N-methyl-D-aspartic acid
BBB - Blood Brain Barrier
LPO - Lipid peroxidase
β-APP - β-amyloid precursor protein
GCS - Glasgow Coma Scale Score
NRAP - National Research Action Plan
TLRs - Toll-like receptors
ICP – Intra- Cranial Pressure
CSF – Cerebral Spinal Fluid
GLAST (EAAT1) - Expression of astrocytic sodium-dependent glutamate transporters
NMDA - N-methyl-d-aspartate
AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazole propionate
mGluRs - metabotropic glutamate receptors
MAPK - mitogen activated protein kinases
NADH - Nicotinamide adenine dinucleotide hydrogen
ROS - reactive oxygen species
mPTP - mitochondrial permeability transition pore
AIF - apoptosis inducing factor
Apaf - apoptotic protease activating factor
BBB- Blood brain barrier
MMP- Matrix metalliproteinases
SOD - superoxie dismutase
GPX - Glutathione peroxidase
4-HNE- 4-hydroxynoneal
3-NT- 3-nitotyrosine
LPO - Lipid peroxidation
CSPGs - chondroitin sulfate proteoglycans
DAI - diffuse Axonal Injury
DMN - default mode network
SN - Sailence network
MES - maximal electroshock seizure
CPSCEA - purpose of control and supervision of experiments on animals
HBSS - Hank’s balanced salt solution
LVG - Local vendor
IAEC - Institutional Animal Ethics Committee
TTR – Time To Right
NADT - novel arm discrimination test
NO - nitric oxide
GSH – glutathione

HE - hematoxyline and eosin
NADT - Novel Arm Discrimination Test
PBS – Phosphate Buffer Solution
TTR - Time To Right

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ABSTRACT:

Pharmacological evaluation of neuroprotective effect of ethanolic extract of roots and rhizomes of *Nardostachys Jatamansi* in experimental animal model of Traumatic Brain Injury

Background and Aim:

Traumatic brain injury (TBI) is multiphased critical neurological condition prompted by mechanical force on head, that advance as disruption to normal brain physiology. Leading to temporary or permanent cognitive, behaviour and physical affliction. TBI pathophysiology is cascade of secondary event after primary injury to head. That includes, damage to blood brain barrier, neuronal inflammation, axonal degradation and generation of reactive oxygen species. *Nardostachys Jatamansi* (NJ) have been found as potent antioxidant, anti-inflammatory agent, promising as neuroprotective effect in neurological condition. The study provides the results of ethanolic extract of *Nardostachys Jatamansi* for TBI induced in rats by Marmarou's weight drop method.

Experimental Method:

In study, Female Wistar rats weighing around 250-400 gm were used. The animals were grouped in 5 groups with 6 animals in each. Groups assigned were Normal control, TBI induced i.e Disease control group, and 3 treatment groups with different doses, namely TBI + NJ 100 mg/kg, TBI + NJ 200 mg/kg and TBI + NJ 400 mg/kg. NJ was given orally once a day for 7 days as pre-treatment before TBI induction. TBI was induced by Marmarou's weight-drop method. The physical parameters were evaluated after 24hr of TBI induction that counts actophotometer, rearing test, beam walk test, rotarod, novel arm discrimination test (NADT) and wire hang test. Lately biochemical parameters were performed after animal sacrifice namely, estimation of Malondialdehyde (MDA) levels, nitrite oxide (NO) and reduced glutathione (GSH) by using half brain of animal. The other half was used to check %water content in brain.

Extraction method: Raw roots and rhizomes of *Nardostachys Jatamansi* was purchased, cleaned and grained to form coarse powder. Soxhlet method was applied using 90% ethanol for 6-8 hour, in 2 cycle at 60 ℃ temperature. The obtained extract was introduced to vacuum

rotary evaporator at low pressure and menstruum was evaporated to obtained remaining residue. The extract was suspended in 0.5% CMC as vehicle. The dose was freshly prepared before oral administration. Extract was stored in cool dark place before use.

Result:

The study found the effect of ethanolic extract of *Nardostachy Jatamansi* is promising as neuroprotective therapeutically in TBI. As NJ ameliorates locomotors and motor co-ordination deficits in treatment group as compared to disease exposed animals. NJ was also able to withstand behaviour and cognitive parameters, evaluated by using actophotometer, rearing and NADT. NJ also significantly improved neuroprotection against lipid peroxidation, nitric oxide by reduced levels and evidently raised reduced glutathione level. The histopathology shows decrease in vasogenic edema and lesion in treated group in contrast of disease manifested group.

Conclusion:

The results signifies the *Nardostastachy Jatamansi* is a potential therapeutic agent for neuroprotection in condition like traumatic brain injury. NJ by acting on secondary cascade of TBI pathogenesis reduces reactive oxidants levels, reduces neuroinflammation, neurodegeneration, edema and allocating as neuro-preserver.

Chapter-1

Introduction

CHAPTER 1: INTRODUCTION

1.1 Introduction:

Traumatic Brain Injury (TBI) is a heterogeneous neurological condition. Owing a leading cause of mortality in developed as well as developing country, the global epidemiology concerns it as silent epidemic while accounting 74 million cases annually worldwide (Nguyen et al., 2016). In addition to this, TBI defect late life quality of patient. TBI is defined as loss of structural integrity of brain, due to shear, mechanical force and rotational force induced by blunt or penetrating injury. Prime visible-physical alternation considered under primary event post trauma are complete or partial level of consciousness, retrograde or post traumatic amnesia, neuronal deficits, change in mental state, other symptoms like dizziness, nausea, movement imbalance and change in motor coordination. When primary events are remained unattended, it initiates cascade of secondary events. That follows neurological conditions at late phase like Alzheimer's, Parkinson, Axotomy ending patient in vegetative state at late life. (Irvine and David Clark, 2018; Pervez, Kitagawa and Chang, 2018). The degree of symptoms can vary and are managed accordingly.

TBI have short as well as long term health condition, that may or may not interfere to daily activities. But has direct or indirect affect on individual's lifestyle. The impact of reasons on epidemiology for injury may vary according to the age group to be evaluated. For stance, elder people (55-80) has high ratio of fall caused TBI so do toddler and preschooler child (1-6 year aged children). Whereas TBI in youngster (18-25 years old) is mostly due to road accident (Gururaj, 2002; Dixon, 2017). Other reasons enumerates sports injury, infection, brain tumour, brain stroke and ischaemic, haemorrhage, etc. (Najem et al., 2018).

Based on clinical features of patient to response, according to the GSC score, TBI can be identified as mild (12.9 score), moderate (12.9-9 score), severe (score is above 9). (Barlow, 2013; Theresa M. Desrochers, Ken-ichi Amemori, 2015; Dixon, 2017; Pervez, Kitagawa and Chang, 2018).

Primary injury is initiated by neuron axonal, oligodentrites and blood vessel damage at multi layer of brain including grey as well as white matter (Mao et al., 2021) TBI may persists from months to years depending on severity neuronal degradation (Ng and Lee, 2019). Secondary TBI is caused by delayed or prolonged molecular & biochemical cascade, neurological or systemic conditions such as haematoma, lesion, axonal disconnection, ionic dysregulation, retraction bulb, vasogenic oedema, haemorrhage, ischaemia, multi-level circuit disruption, a synaptic loss followed by

oxidative stress, neuroinflammation, and neurodegeneration. Damage to brain blood barrier plays vital role is activation injury. Main pathophysiology of TBI includes free radicle generation and reactive oxygen species causing oxidative stress, increased intracranial pressure due to excitotoxicity, neuroinflammation and mitochondrial dysfunction. Activation of these events cause burst of cytokines, inflammatory mediators, that further cause neuronal damage, axonal degeneration and programmed cell death, impairment of autophagy and lysosomal pathways, glial scar and myelin-associated axonal growth inhibitors realease.(Prins et al., 2013; Ladak, Enam and Ibrahim, 2019)

Excitotoxicity is result of over expressed release of excitatory neurotransmitters mainly glutamate and N-methyl-D-aspartic acid (NMDA). Over activation of glutamic receptors cause ionic influx of calcium and sodium (Wang, Cui and Gao, 2016). Imbalance of ion homeostasis cause seizure like worsen condition in patients. Increased in calcium intracellular level leads to activation of catabolic enzymes that corrupts mitochondrial membranes (Floyd, Gorin and Lyeth, 2005). Giving rise to energy demand and shifting to pyruvate cycle instead of glucose cycle forming lactic acidosis. Astroglial activation is key contributor to glial scar formation that activates inflammation and unregulated neurotoxin level causing uncontrollable situation (Kumar and Loane, 2012)

Neuroinflammation is indispensable part of TBI and imply astrocyte, residential microglial cells and cytokines worsening situation while working with restorative process of brain, by dual effect of inflammation. Inflammation is commenced by complement activation or microglial activation. Storm of cytokines and microglial activation, increase influx of immune component in BBB damaging (Bellander et al., 2001; Fluiter et al., 2014). Microglial activation shows 2 primarily phenotype M1 and M2. Balancing for and against effect showcasing dual effect of inflammation. Where M1 phase is conventionally activated and responsible for neuronal death releasing IL-1, TNF, IL-6 and IF. While M2 is alternatively activated responsible for rehabilitation action after inflammation for protective effect and releases anti-inflammation cytokines IL-10 and IL-4 (Morganti, Riparip and Rosi, 2016).

Extended duration of microglial cells and inflammatory cascade sources degeneration of neuron by increased level of tau protein, directly correlating to Alzheimer's. Increased level of LPO- Lipid peroxidase encouraging SOD-1 activity promising relation of oxidative stress in Alzheimer development (Ramírez et al., 2005; Cherry et al., 2016). TNF- Alpha is vital proinflammatory

cytokine extending damage; over expression indulge long-term effect for namely post TBI seizure due to abnormal brain excitation (Turtzo et al., 2014)

Axonal degeneration: Diffusional axonal injury gives rise to wallerian degeneration within few hours of injury. The mechanical impact cause disruption and damage to cytoskeletal axonal networking. Simultaneously, constant proteolysis mediated by calcium and axonal damage, cause axotomy (complete physical break down of an axon). This is further proved by hall marker formed by retraction bulb formation, namely neurofilament and β-APP (β-amyloid precursor protein) that indulge axonal swelling, damage to oligodendrocyte, deformation of white matter and apoptotic cell death of neuron (Povlishock, 2006)(Hellewell et al., 2010).

Autophagy and lysosomal pathways plays important role in maintaining cell homeostatis, protection, and stability by removing intracellular protein formed during stress. Tough role of autophagy is controversial for detrimental or beneficial role. But due to increase in lysosomal permeability, autophagic impairment and increased concentration of autophagosomes is observed. Exaggerating traumatic event by neuronal death (Taylor et al., no date).

1.2 Hypothesis:

TBI is worsening mostly by secondary molecular and biochemical events. Reversing primary injury is not possible, but as secondary injury is emerged from primary injury, there is chance to be managed with supportive care or targeting pathways. TBI being **multi pathed condition**, working on specific single targetted pathway at a time of injury may not give desired outcome. *Nardostachys Jatamansi* (NJ) has been reported to function in neurological conditions since ages. Main constituents of *Nardostachys Jatamansi* thought to be showing effect is sesquiterpenes. There are more then 12 bioactive sesquiterpenes in NJ. Amongst them Nardosinone and desoxo narchinol are found to be predominantly effective as anti-inflammatory by decreasing LPS induced Nitric oxide overproduction in studies. Additionally this compounds also reticent proinflammatory mediators and cytokines that includes IL-6, IL10, TNF, PGE and iNOS. This proves Nardosinone type sesquiterpenes as antioxidant and neuroprotective agent. Hence, working on overall condition **simultaneously** may becomes therapy for TBI. We hypothesized, ethanolic extract of *Nardostachys Jatamansi* with neuroprotective, anti-inflammatory and anti-oxidant properties to become potential therapy for TBI.

1.3 Objective:

1. To evaluate effect of ethanolic extract, of *Nardostachys Jatamansi* extract on experimental model of traumatic brain injury.

2. To investigate possible mechanism of action *Nardostachys Jatamansi* as treatment of TBI.

Chapter-2

Review of Literature

CHAPTER 2: REVIEW OF LITERATURE

2.1 INTRODUCTION:

Traumatic brain injury (TBI) is the term used to describe brain damage brought on by an abrupt, external mechanical force applied to the head. The head injury penetrates deeper inside the brain to white matter and degenerates neurons. This neurodegeneration leads to short-term or permanent cognitive, physical and psychological impairment along with diminished or alteration in consciousness. The frontal and temporal areas of the brain are particularly involved in TBI. Brain injury is considered to be very crucial because of the intricacies associated with the structure and functions of the brain. It is estimated that every year around 50 million individuals are affected by TBI (Ghaith et al., 2022). Laceration and bruising on the basal surface of the skull is the result of these injuries. Often bleeding in the internal parts of the brain and deficiency in the amount of oxygen occurs in the brain due to injury. When the damage is mild connectivity between axons is disrupted (Williams et al., 2018). Classification of TBI into mild, moderate, and severe injuries is performed using Glasgow Coma Scale Score (GCS) (Vella et al., 2017).

Due to increased rates of cases and awareness at international level, many steps were put forward to understand molecular basis as well as management of TBI at greater extend. Involvement of the Departments of Defence, Veterans Affairs, Health and Human Services, and Education to develop a National Research Action Plan (NRAP) is also seen. After several researches, the clinical diagnosis is still a concern for disease management. But to confront injury of human brain at emergency department of hospital, physical assessment can be done that also includes cognitive function to standardize these finding, Glasglow Coma Score is used. That also determine severity of injury. Simultaneously, for precise understanding imaging like MRI, CT scan is performed. The other developed methods are Checking intracranial pressure, cerebral flow, Collection of bio specimens like protein and messenger RNA expression by doing autopsy. (Ng and Lee, 2019)

Most frequently used, GCS is the best option to examine the motor, vocal, and ocular responses of TBI patients. If a patient is having GCS score of 13-15 he is considered of having a mild injury, similarly, for moderate injury, the score lies between 9-13 and 3-8 for the severe injury. (Mena et al., 2011) . Post-trauma if the injury arises immediately is termed as primary injury and secondary trauma arises when there is an occurrence of molecular and cellular reactions which lasts for a longer time. There are a set of symptoms that are noticed due to mild injury which includes disorientation, dizziness, nausea, and balance problems. As the case worsens imbalance in

locomotory activities, vision change, and aphasia are seen (Pervez, Kitagawa and Chang, 2018). Some biochemical, molecular, and physiological changes occur after an initial injury. Various cascades like neuroinflammation, the release of pro-inflammatory cytokines, cell death, glial cell activation, and cerebral oedema, contusions all take place (Ladak, Enam and Ibrahim, 2019). A severe brain contusion is frequently accompanied by an initial period of unconsciousness, which is usually followed by recovery. Oedema can result in seizures, changes in awareness, or localized neurologic symptoms. Surgery is infrequently performed for cerebral contusions. Mechanical injury is the causative factor for primary injury; secondary injury arises due to initial injury. Damage to brain and surrounding tissues is the result of several changes including elevation in intracranial pressure, haematomas and edema (Huffman et al., 2010). Assessment of intracranial pressure can be done by MRI scan (Dixon, 2017). Medical interventions mainly focus on targeting secondary injury. Managing TBI can result in noticeably better outcomes, including improved functional outcomes scores and decreased mortality rate, length of hospital stay, and costs (Xiong, Mahmood and Choop, 2013). Several targets improve brain injury that involves decreasing brain swelling, and use of neurodegenerative. Toll-like receptors (TLRs) blocking is another approachable target to treat TBI. TLRs are involved in detecting TBI-related damage and triggers neuroinflammation by releasing a variety of cytokines and chemokines as an immunological reaction to defend the body from encroaching pathogens. Since various cascades are involved in the pathogenesis of TBI, more therapeutic approaches are the need of an hour to decrease the morbidity and mortality associated with the disease (Braza et al., 2016).

2.2 EPIDEMIOLOGY:

TBI is having mortality and disability rates worldwide. TBI is the leading cause of deaths in the population of age between 1-44 years. Fall and road accident are the major cause for TBI. Prevalence of disease is 6 times higher in male than female. In the US 1.7 million of cases were reported annually in 2002-2006. 30.5% of mortality rate is noted every year because of TBI (Faul and Coronado, 2015). Annual cases of TBI-related deaths in the USA was 61,000 in 2017 and, 64,362 in 2020 according to CDC. Whereas, India has 100,000 TBI- death cases per year. According to data of 2019- 2020 year, estimation counts around 611 TBI-related hospitalizations and 176 TBI-related deaths per day. As the prevalence of the disease is high , economic burden of the country also increases. United states spend $ 4 billion annually to treat TBI (Etc and Das C, Lucia MS, 2019) all causes and levels of severity, is 939 instances per 100,000 people, or 69 million people. Mild TBI affected 740 cases per 100,000 persons, or approximately 55.9 million

people. Severe TBI affects 5.48 million people, with 73 cases per 100,000 people (Dewan et al., 2019).

Many cases of the TBI remain unreported or unnoticed. Around 1650 teenagers have concussion which is not reported. As a result, TBI is a “silent epidemic”. Epidemiological survey, prevention, treatment and care should be initiated to lower the incidence of the disease, since many number of cases with injury are higher than reported (Nguyen *et al.*, 2016).

2.3 GLASSGOW COMA SCALE:

Treatment of TBI highly depends on diagnosis and GCS score of the patient, that determines level of care and type of treatment to be given. GSC is Glasgow Coma Scale use to determine level on severity of injury. Score is purely based on response of patient after injury towards external stimuli, clinical symptoms seen. High score indicates less severe injury. Main 3 category is mild, moderate and severe TBI.

Glassgow coma scale was published by University of glasgow professor Graham Teasdale and Bryan Jennett in 1947. Scale determines consciousness of patient exposed to trauma or acute medical condition, perhaps score is subjective in nature. The main 3 compartment of score are: motor response(M), eye moment(E), and verbal ability(V). Each score is individually measured and clubbed later to form total Glasgow Coma Score forming overall severity check.

The score is described as GSC(total score)= E(score of eye movement) V(Verbal ability score) M(motor activity). The value stands between 3 to 15 where 3 is worst and 15 showing best condition. (Teasdale and Jennett, 1976; Buck, 1999).

2.4 TYPES OF TBI:

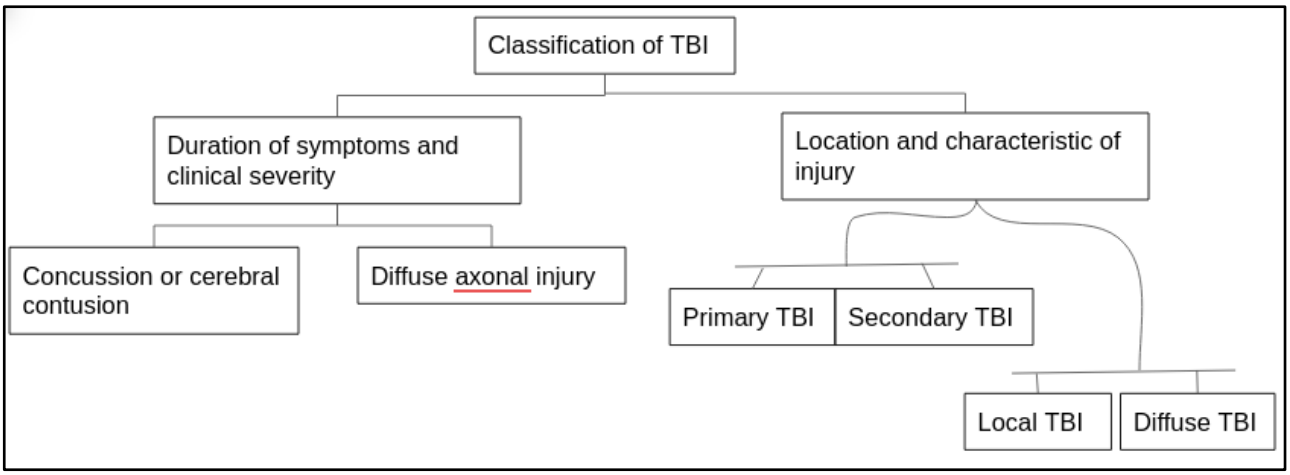


Figure 2.1: Classification of TBI.

(Menon et al., 2010)(Barlow, 2013; Theresa M. Desrochers, Ken-ichi Amemori, 2015; Pervez, Kitagawa and Chang, 2018)

Mild TBI: Refers to injury caused by blunt object or acceleration and deceleration action, usually it is closed type injury. Patient goes through disorientation, fatigue, irritation, headache in some cases unconsciousness, attention deficiency and loss of memory is also observed. This may range from 6 month to 1 year. Physician treat mild injury by suggesting full bed rest and supportive care. Although mild injury is treatable, it shows post-concussive syndrome in 30-53% of patient. Mild injury shows 13-15 GCS score.

Moderate TBI: It is considered when GSC score is between 9-12.9. This kind of injury happens because of falls, vehicle collision, road accident. Clinical symptoms manifest behavioural and cognitive impairment and physical balance loss. Such injury may lead to serious contusion, or haemorrhage and is admitted to ICU. Where surgical interventions is done maintaining intracranial pressure, removing blood clots to treating skull fracture. Simultaneously oxygen is supplied while medication includes painkiller, anticonvulsant drugs, sedatives and hypotensive drugs in case of prophylaxis treatment.

Severe TBI: GSC score less than 9 indicates severe TBI, where patient is under critical condition, being vegetative for long period of time. Showing absence of physical and intellectual dysfunction.

Glassgow Coma Scale

Table 2.4.1: Glassgow Coma Scale assessment table.

	Response	Score
Best Eye Response (E)	No eye opening	1
	Eye opening to pain	2
Total Score= 4	Eye opening to sound	3
	Eyes open spontaneously	4
Best Verbal Response (V)	No verbal response	1
	Incomprehensible sounds	2
Total Score=5	Inappropriate words	3
	Confused	4
	Orientated	5
Best Motor Response (M)	No motor response.	1
	Abnormal extension to pain	2
Total Score= 6	Abnormal flexion to pain	3
	Withdrawal from pain	4
	Localizing pain	5
	Obeys commands	6
	TOTAL SCORE	**15**

Adapted from Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet 1974;2(7872):81–4

2.5 CURRENT TBI MANAGEMENT:

The conventional treatment of TBI is as follows:

1) Ventilation treatment: Metabolic processes and energy generation is highly dependent on supply of oxygen through blood. Due to haematomas and clotting ischaemic condition is seen in brain post injury. To increase survival rate of neuronal cells supply of oxygen should not stop. Hence ventilation whether be mechanical or intubation is provided as symptomatic treatment in TBI.

2) Hyperosmolar treatment: due to inflammation and other immunological events after TBI according to pathophysiology, fluid is accumulated causing intensive pressure. That is balance by hyperosmolar agent helping expels fluid out of the body.

3) Prophylactic hypothermia: It helps indirectly to prevent death and cardiac dysrhythmia.

4) De compressive Craniotomy: craniotomy is surgical procedure to remove part of skull, this method is performed when intracranial pressure is uncontrollable and sudden reduction of ICP is strongly required. This will help prevent herniation and haemorrhage.

5) Sedatives and painkillers: Overall management of stress, intracranial pressure and conditioning patient to bare pain is needed for treatment and aiding the condition.

6) Steroids: works by supporting vascular strength meanwhile also drop neuroinflammation by reducing generation of free radical.

7) Antibiotics: Pathogen invasion is very easy when body is busy fighting and maintaining critical cascade caused by injury. To decline infection of microbes, anti-infective is compulsorily prescribed.

8) Anticonvulsant: Convulsion is commonly seen post TBI condition due to glutamic signal fire. That may be due to excitatory mechanism and increase in intracellular calcium ions. Anti convulsion will help to maintain the condition and lower the chances of convulsion. (Lucke-Wold et al., 2015; Dixon, 2017; Dash and Chavali, 2018)

2.6 PATHOPHYSIOLOGY:

TBI is multidirectional neurological condition broadly classified into 2 stages: primary traumatic brain injury and secondary injury which is cascade of primary injury if remains unattended. Primary injury is initiation of cascade caused by physical external force forming diffused of focal injury to the brain (Ng and Lee, 2019). It can be due to counter-current force, rotational force of direct blunt or penetrating injury to the brain. Focal injury is also known as extraxial force, i.e. out side of the skull parenchyma, which may lead to subdural haematoma, epidural haematomas, subarachnoid haemorrhage (Capizzi, Woo and Verduzco-Gutierrez, 2020). It includes damage to the structure of brain forming blood vessel disruption, neurons, axons, dendrites, glial cells, endothelial damage that may include skull fracture (Barlow, 2013). Secondary injury initiates as response to the brain tissue damage, main pathophysiology playing role id formation of oxidative stress, neuroinflammation, and lastly neurodegeneration.

As TBI is characterised by heterogenic condition, all the pathways initiate together or individually leading to mild, moderate and severe injury. Because of brain integrity damage, a quick response like impairment of neuronal network, damage to tight junction protein, leakage of CSF and activation of inflammatory cascade (Bryant et al., 2015; McGinn and Povlishock, 2016; Dixon, 2017; Pearn et al., 2017; Ladak, Enam and Ibrahim, 2019; Amyot et al., 2020)

Shearing injury also known as diffusive brain injury is cause by dispersed degeneration of neurons. Which is also known as cerebral white matter degeneration.

Secondary brain injury, that cause more severe mechanical damage to cerebral of brain which was caused by initial injury insult. It cause ionic disturbance, excitotoxicity, mitochondrial dysfunction, reactive oxidative species generation, neuroinflammation and eventually neuronal cell death (Ng and Lee, 2019; Baig and Sanders, 2020) (Tran, 2014).

Pathways contributing to secondary injury:

2.6.1. Excitotoxicity

2.6.2. Ionic disturbance

2.6.3. Mitochondrial dysfunction

2.6.4. Neuroinflammation

2.6.5. Oxidative stress generation and lipid peroxide formation

2.6.6. Glial Scar and Myelin-Associated Axonal Growth Inhibitors

2.6.7. Axonal degeneration

2.6.8. Apoptotic cell death

2.6.9. Autophagy and lysosomal pathway.

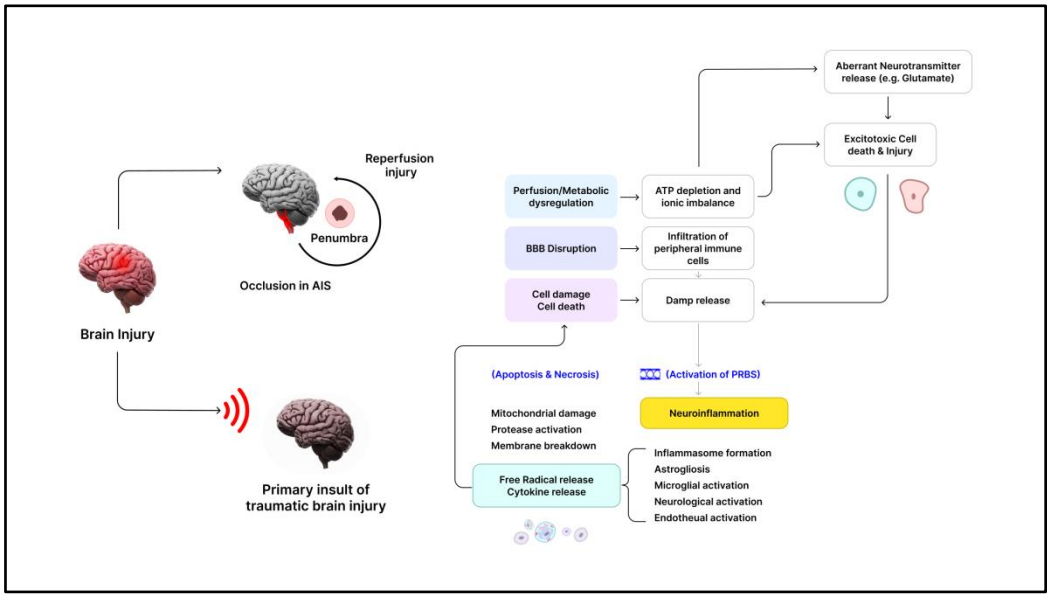


Fig: 2.6.1: Secondary pathophysiology of traumatic brain injury.

2.6.1. Excitotoxicity:

Excitotoxicity is prime pathway for secondary injury cascade. Primary neuronal damage or death demonstrates BBB leakage, that altogether spurse excessive excitatory amino acids from synapse in cortical and hippocampal regions. That further contributes to glutamate re-uptake failure by dysfunctionality of glutamate transporter. Due to over saturation of excitatory amino acid the two receptors namely: Expression of astrocyte sodium-dependent glutamate transporters GLAST (EAAT1) and GLT (EAAT2) stop functioning. Eventually results in activation of glutamate receptors, of iGluRs family: N-methyl-d-aspartate (NMDA) receptor and α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA). (Monsour, Ebedes and Borlongan, 2022) States long term alteration causing seizure like condition ultimately effecting cognition and memory.

iGluRs receptors are membrane depolarizer, ligand gated ion channel transporting Na+, K+ and Ca2+ ions on glutamate binding while metabotropic glutamate receptors (mGluRs) regulating Ca2+ and downstream signalling by GTP- binding protein. Over activation of NMDA and AMPA receptor alters influx of Ca2+ and Na+ ions from extracellular matrix. That triggers multiple downstream signalling pathway molecules like, Ca2+/calmodulin-dependent protein kinase II,

mitogen activated protein kinases MAPK. The whole cascade further retain calcium at postsynaptic neurons giving rise to reactive oxygen species detriment secondary injury.

mGluRs activates phospholipase C/ inositol- 1,4,5- triphosphate that liberate intracellular stored calcium forming more Ca2+ accumulation. Resulting in apoptotic cell death activating proteins like Caspases, Calpin and Calcineurin. Concluding to interupted homeostasis and mitochondrial damage. (Ng and Lee, 2019)

2.6.2. Ionic disturbance:

Due to trauma to the brain, condition like hypoxia and hypertension is observed, that may be derived from focal and diffusive injury. This leads to imbalance of ions, that affects cellular metabolism of oxygen as hypoxia is condition forming low level of oxygen in tissues. Deficit of oxygen level, glycolytic process shifts to pyruvate cycle. The shift cause formation of lactate from pyruvate ending in lactic acidosis. Furthermore, it also increase Nicotinamide adenine dinucleotide hydrogen (NADH) level. This develops energy demands as ATP synthesis is harshly declined forming on 2 ATP instead of 32 ATP in anaerobic condition. This directly increase sodium level in cell because of down regulated NA+/K+/ ATPase pump. Indirectly also influence Na+/CA+ and Na+/H+ pump elevating calcium entry and excessive intracellular level of calcium. In presence of calcium glutamate signalling is disturbed forming more cellular damage and mitochondrial damage.

2.6.3. Mitochondrial dysfunction:

Mitochondria being important player in TBI pathophysiology, it is cell organelle also known as power house of the cell. Functioning as Energy generator by forming ATP out of digested food by several enzymatic process like; Krebs's cycle, amino acid metabolism, and fatty acid b-oxidation. (Hiebert et al., 2015) Apart from this, Mitochondria also serves as neuronal cell homeostasis maintainer, by myriad of intricate processes. Out of them autophagy is a part, that eliminate metabolic by-product ROS and Ca2+ causing damage.The dis-functioning of mitochondria start post 30 mint of TBI. Structurally, mitochondrial affects it’s efficiency depending on cristae, a multifold structure extended to inner membrane of mitochondria, Swelling or structural damage to cristae cause membrane potential loss. Mitochondrial dysfunction serves as hallmark for TBI. The Ca2+ and other ion accumulation into mitochondria produce ROS, depolarization, stop generating ATP. That breakdown Electron Transport Chain eventually, imparing oxidative phosphorylation,

calcium cycle and disturbed metaboli restoratio and activation of mitochondrial permeability transition pore (mPTP).

All the pathology cause release of mitochondrial proteins: cytochrome C and apoptosis inducing factor (AIF). Cytochrome C is released by mitochondria to outer part of the mitochondria and binds to apoptotic protease activating factor (Apaf-1) and ATP, and forms complex called apoptosomes. Apoptosome activates Caspase 3 by cleaving procaspases. Initiate apoptotic mechanism by generating TNF by macrophage activation, by extrinsic mediator of apoptotic pathway.

2.6.4. Neuroinflammation:

Neuroinflammation is key aspects that demonstrate as dual functions i.e neurotoxin as well as neuroprotective in traumatic brain injury depending on the time passed in injury (Schmidt *et al.*, 2004). Neuroinflammation is inflammation inside the brain mediated and controlled by residential microglial, leukocytes and astrocyte (Morganti, Riparip and Rosi, 2016; Ladak, Enam and Ibrahim, 2019). Inflammation can be mediated through cytokines, intracranial complement activation, post traumatic ischaemia and oxidative stress (Schmidt et al., 2004). BBB- Blood brain barrier is protective structure that differentiate and permits exchange of certain ions, nutritive and chemicals between neural and blood factors. It helps to balance brain homeostasis. BBB disruption or damage to tight junction cause larger molecules like proteins and serum to enter the brain. Entry of leukocytes, release MMP- Matrix metalliproteinases in response causes brain edema.

(Postolache et al., 2020). Due to presence of TNF-α, it closely interacts with ligands from Fas family initiating caspases leading to programmed cell death. Eventually accumulating more leukocytes at injury. Detained and extended neuroinflammation recruits microglial, macrophages further causing astrogliosis overall worsening neuroinflammation (Gentleman et al., 2004; Johnson et al., 2013).

2.6.5. Oxidative stress generation and lipid peroxide formation:

Many research have suggested, Oxidative stress playing huge pathogenesis role in secondary event of TBI. Oxidative stress leads to oxidative damage. The atoms or molecule with unpaired electron are termed as free radicals, The unstable molecule with oxygen molecule is termed as reactive oxygen species. This reactive free radicals readily damage the body, forming ROS. To prevent free radical damage body consists antioxidants that works by reacting with free radicals to prevent binding with other vital molecules in cell and inhibit dominoes of oxidative damage. The two

natural antioxidant enzymes are superoxide dismutase (SOD) and Glutathione peroxidase (GPX). (Cornelius et al., 2013; Keshavarzi et al., 2021) In TBI, due to Ca2+ accumulation, enzymatic reactions, mitochondrial damage, excitotoxicity and activated innate immune cell production of nitric oxide and reactive oxygen species is boosted. Generation of ROS/RNS develops oxidative and nitrosive stress causing damage to lipids, nucleic acids and proteins.

The main oxidant performing TBI pathogenesis are superoxide anions, hydrogen peroxide and hydroxyl radicals. Which can be treated by using either approach: (i) taking down ROS/RNS (ii) abounding production of oxidative stress or (iii) by reacting with metal ins for ROS generation catalysis.

Superoxide radical: It is microvascular radical. The sourcing of superoxide can be due to numerous reasons post TBI such as arachidonic acid (AA) cascade enzymatic, mitochondrial leakage, haemoglobin oxidation, activated microglial and neutrophils-macrophage infiltration. With help of enzyme super dioxide dismutase (SOD) react with O2c- (superoxide) to form more reactive H2O2.

Lipid peroxidation: it's oxidative process of lipids, where electrons are taken from lipids present in cell membrane eventually causing cell membrane fluidity, permeability and lowering ATPase leading to cell membrane damage. Fatty acid is one, forming fatty acid radical. Being unstable molecule it reacts with oxygen molecule creating peroxyl-fatty acid radical that again produce that is called as lipid peroxide. The reaction is divided in 3 phase of oxidation: initiation, propa- gation, and scission. The last phase scission give rise to aldehydes 4-hydroxynoneal (4-HNE) or 2-propenal (acrolein). Increase in 4-HNE or 3-nitotyrosine (3-NT), impares synaptic plasticity in hippocampus and cortex region of brain. ROS and LPO are associated with cerebral flow blow, immunosupression, plasticity post TBI.

2.6.6. Glial Scar and Myelin-Associated Axonal Growth Inhibitors:

Axonal degeneration is commonly seen in TBI, that is resulted from astrocyte activation triggered by TBI. The activated astrocyte infiltrate to lesion site turning into intermingle of astrocyte with oligodendrocytes, microglia, fibroblast and meningeal cells developing as scar in brain. Other factors affecting axonal regeneration are neurone and versican in glial scar known as chondroitin sulfate proteoglycans (CSPGs) and inhibitory molecules like tenascins and semaphorin 3A. These factors by down regulating RhoA activity leads to collapse by inducing growth cone.

2.6.7. Axonal degeneration:

Post Diffuse Axonal Injury (DAI), mechanical injury disturb axonal structure and network causing damage. Axonal damage can be seen as swollen axon, cytoskeletal breakage. Main factor responsible for DAI is calcium ion accumulation that degrade myelin sheath, damaging signal transport. Due to accumulation of transport materials like protein, axon retract itself back forming retraction ball. The Wallerian degeneration is observed near breakdown point. The damage can be distinguish as Bulb formation another one is microtubule breakage. Retraction bulb formation is responsible for function loss. Whereas microtubule breakage is termed as axonal varicosity. Neuronal networks are of 2 set, the default mode network (DMN) and Sailence network (SN). Both the network is responsible for autonomous and cognitive activity. But due to DAI, DMN is affected and cannot communicate with SN showing cognition deficits. The DAI can be detected by hall markers- beta amyloid precursor protein and neurofilament.

2.6.8 Apoptotic cell death:

Apoptotic cell death is programmed cell death functioning as various physiological processes such as, tissue homeostasis, development and removal of unwanted or mangled cells. Apoptosis displays morphological and biochemical characteristics like cell shrinkage, membrane blebbing, chromatin condensation, DNA fragmentation and formation of apoptotic bodies. Activation of programmed cell death involves 2 important molecules namely pro apoptotic and anti-apoptotic. The pro apoptotic factors like cytochrome C is released by mitochondrial as intrinsic pathway leading to activation of cysteine proteases like caspases and calpain. Whereas the extrinsic pathway includes TNF and Fas binding to their specified cell surface receptors. Both the pathway further downstream caspase dependent signaling by activating caspase 8 and 9. That eventually cleave while activating caspase 3. On the other side, there is burst of mitochondrial proteins namely Smac/ DIABLO, poly (ADP-ribose) polymerase-1, AIF and endonuclease G are responsible for DNA fragmentation by translocating to nucleus and activation of downstream signalling molecule. The pathway is caspase- independent apoptosis activated through proteolysis of cytoskeletal protein by calpains. The pathway is responsible for neuclic acid damage, condensation of chromatin in glial as well as neuronal cells. (Ng and Lee, 2019)

2.7 DRUG:

2.7.1 *Nardostachys Jatamansi*:

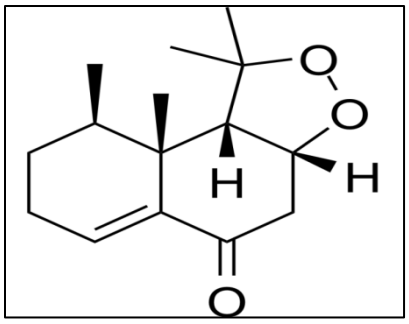
Nardostachys Jatamansi is Himalayan plant belonging to *Valerianaceae* family found to be a potential medicinal plant in many medicinal plants (Bhattacharya and Dhiman, 2020). The plant is pharmacognostically identified as long stout of 10-60 cm in height with woody root stock. The leaves are, Flower are Roots are Rhizomes are (Ahmad et al., 2013)

Fig: 2.7.1(A): Raw roots and rhizomes of Jatamansi

Plant is found in Bhutan, India, China, Nepal and Tibet at 2200m to 5000m altitude (Sahu *et al.*, 2016). Jatamansi is well recognized by the modern to folk medicine system. NJ is penned as tapaswani, balchara, bhutijata, jatamaschi, manshi and bhytajata in India and musk root, spikenard, Balchad and Jatamanson internationally. (Purnima and Kothiyal, 2015). The use of NJ is dated back to Vedic era. And it is been used since, in many folk medicinal system like Ayurvedic, Nepali and Kempo. In world of allopatheic and synthetic medicine, people from native of NJ still priortize use of NJ in medical conditions. (Rokaya, Münzbergová and Timsina, 2010) **Rokaya et al** described use of NJ in cough, headache, poisioning, leprosy, infection traditionally. In addition, it is been studied for certain promising pharmacological effects like antioxidant, hypolipidemic, hepatoprotective, Cardiac tonic and certain neurological condition like, anticonvulsant, antiparkinson and anti alzheirmer's. (Sahu *et al.*, 2016) It's is known for antihypertensive, anti arrhythmic anti-inflammatory, antioestrogenic, antiasthmatic, anti tumor (Singh *et al.*, 2013).

Nardostachys Jatamansi consists of alkaloid, sesquiterpenes and flavonoid as it's chemical constituent. From which Sesquiterpene is most abundant and responsible for neurological aid (Yoon *et al.*, 2018). Amongst more than 12 sesquiterpenes, Nardosinone, Desoxonarchinol, Kanshone, Jatamasone, nardostachone and Narchinol showcasing neuroprotective effect in several in-vivo experiments are found predominantly (Singh, Kumar and Duggal Sanjiv, 2009).

Nardosinone is principle sesquiterpene hinged in *Nardostachys Jatamansi* (NJ). Is proven by comparing effect with whole plant. Hence also, observed as quality checker of plant in Chinese medicine.(Wen *et al.*, 2021) Nardosinone or nardosinone like compound are white crystalline powder in appearance, with great solubility in methanol and insoluble in ether (Rehman and Ahmad, 2019)



Nardosinone: Chemical structure

Chemical formula: C15H22O3

Fig 2.7.1(B): Chemical structure of Nardosinone sesquiterpene dervied *from Nardostachys Jatamansi.*

2.7.2 Bioactivity of Nardosinone:

Neurological action:

(Sachin Parmar, Amit Gangwal, 2011)conducted experiment on sleep deprived depressed animals, and concluded dose dependent elevated effect of methanolic extract on force swim test and tail suspension test for animal behaviour study. Showcasing positive usage of NJ in patient suffering with sleep disorder as well as depression. (Rao, Rao and Karanth, 2005) on other hand experimented anticonvulsant and neurotoxicity effect on maximal electroshock seizure (MES) model and PTZ induced seizure model. Where ethanolic extract worked efficiently on MES model while no effect on STZ induced seizure. Challenging further investigation on the results. (Liu *et al.*, 2018) also explained ethanolic extract of NJ showed neuroprotection against beta amyloid toxicity in Alzheimer's disease in both in vitro as well as in vivo experiment. Where mechanism shows reduction in glial cells, level of free oxidative radicles, and inhibitory action of extracellular-signal- regulated kinase phosphorylation, providing anti-inflammatory and antioxidant effect. (Salim *et al.*, 2003) examined effect of *Nardostachys Jatamansi* on middle brain occlusion model of Acute ischaemia in male wistar strain rats. The study confirmed virtue of antioxidant property

exhibited by alcoholic extract of NJ. When 15 days pre treatment was given it shows visible protection enhancement against ischaemia, probably by intensifying function of GABA-Glutamergic Amino Butyric Acid and decreasing excitotoxicity by hyperpolarisation, which exactly happens during secondary mechanism of TBI.

For centuries, researchers have been assessing the anti-inflammatory properties of Nardosinone and the entire *Nardostachys Jatamansi* plant. These investigations have unveiled encouraging outcomes, prompting further exploration into the potential molecular mechanisms underlying their actions (Kim *et al.*, 2021). Their actions were elucidated through the downregulation of mRNA overexpression of pro-inflammatory factors like IL-1B, IL-6, and iNOS. Additionally, they inhibited LPS-induced inflammatory mediators in both in-vivo and in-vitro experiments. Chemical analysis revealed the prominent presence of desoxo-narchinol A, a sesquiterpene compound found in *Nardostachys Jatamansi* (NJ).

Chapter-3

Materials and Methods

CHAPTER 3 MATERIAL & METHODS

3.1 PROTOCOL:

The protocol of the experiment was approved by institutional animal ethics committee as per the guidance of the committee for the purpose of control and supervision of experiments on animals (CPSCEA), ministry of environment, forest and climate change, Government of India. Protocol number is IP/PCOL/MPH/32/2022/05.

3.2 DRUGS AND CHEMICALS:

0.1% CMC, 99.8% ethanol, Distilled water, thiobarbituric acid were purchased from Sigma Aldrich (USA) and Hank's balanced salt solution (HBSS) was purchased from thermofisher. Dried raw root of *Nardostachy Jatamansi* was purchased from LVG (Local vendor) in Ahemdabad, Gujarat. Roots and rhizome was authenticated by a prominent botanist affiliated to Gujarat University (Ahemdabad, Gujarat).

3.3 EXTRACTION METHOD:

Dried raw roots and rhizomes of *Nardostachys Jatamansi (NJ)* availed from local vendor. Further it was identified and authenticated by botanist of department of botany at Gujarat University. Dried roots and rhizomes were cleaned and grinded to form coarse powder. Ethanolic extract was prepared as indicated by (Prabhu, Sudhakar Karanth and Rao, 1994). 200 gm of coarsely powdered roots and rhizomes was extracted for 8 hr at *60 °C.* Extraction was carried out by using soxhlet apparatus with 90% ethanol as solvent. Obtained extractwas evapourated at reduced temperature and pressure with help of rotatory vacuum evaporator to obtain dry extract (Radhakrishnan et al., 1998). Obtained dried extractwas weighed and stored at 4 *°C*. The 8% yield was obtained from crude drug. NJ extract was lastly suspended in 1% CMC to prepare oral NJ extract dose. (M. et al., 2013, Bährle-Rapp, 2007)

3.4 ANIMALS PROCUREMENT AND CONDITIONING:

30 Female Wistar rats weight around 250- 350 gm were used for current study. 3 Rats were caged in each polypropylene cage. Animal house was equiped with air conditioner with 10% air exhaust allowing ideal animal environment. Additionally, rooms were humid at 60±5% while temperature was 25 ± 3˚C. Proper sleep-wake cycle of 6 hours was maintained to fullfill compliance of CPCSEA guidelines. Animals were allowed to access unlimited food and water till the end of

project. Protocol of project was accepted by Institutional Animal Ethics Committee (IAEC) as protocol ID IP/PCOL/MPH/32/2022/05. Animals were acclimatized for 14 days before starting the project and were divided in 5 groups having 5 rats in each group.

3.5 DISEASE INDUCTION MODEL: Weight drop method:

Weight drop method was used to induce close head diffusional injury. As procedure 40 mg/kg of thiopentone was administered in animals through *i.p* in animals (rats). Once animals were anaesthetized they were placed in prone position on sponge bed of 6 cm thickness. 450 gram weight was tied firmly with string. After positioning animal on bed, rat head is covered with steel cap and weight is dropped on the cap, to circumvent direct head injury. Rat was introduced to the weight dropping in torso down position. Later cotton with topical lidocaine was applied on rat's head. The rat was allowed to be in supine position and time to right (TTR) or time to gain consciousness was recorded considering as time to response. Other typical behaviour was also observed in cage such as grooming, ability to walk, exploratory behaviour until the normal pattern was observed (Virginia, 1994, Marmarou et al., 1994).

3.6 EXPERIMENTAL DESIGN:

In this study, animals were assigned into five groups: Normal control, TBI Induced, 3 Pre-treated groups with different doses of NJ; TBI + NJ 100 mg/kg, TBI + NJ 200 mg/kg and TBI + NJ 400 mg/kg. Six animals were kept in each group. Pre-treatment of *Nardostachys Jatamansi* ethanolic extract was given for 7 days orally once a day. Control group was introduced by vehicle. While drug was given according to group as discussed.

Behavioural assessments were conducted on all animals to assess any interference with locomotor and neurological functions caused by the drug in traumatic brain injury (TBI). In each group, behavioural parameters were observed following the trauma, which was induced using Marmarou's weight-drop method. After a 24-hour post-trauma interval, the behavioural assessments were repeated, and then the animals were euthanised for further biochemical and histopathological analysis.

The behavioural assessments included tests such as beam walking, rearing, actophotometer, wire grip strength, rotarod, and the novel arm discrimination test (NADT). Brain tissue was isolated for biochemical analysis, which encompassed the determination of brain water content, levels of lipid peroxidation (LPO), nitric oxide (NO), catalase, and reduced glutathione (GSH). To investigate

brain histology and structural differences, hematoxylin and eosin (HE) staining was performed for histopathological studies.

Table 3.6.1: Animal grouping for experiment.

Group Number	**Group**	**Number of Animals**
1	Normal Control	6
2	Traumatic brain injury (TBI)	6
3	TBI+ NJ Dose 1 (100mg/ kg)	6
4	TBI+ NJ Dose 2 (200mg/ kg)	6
5	TBI+ NJ Dose 3 (400mg/ kg)	6
	Total	30
	Total animals 60	

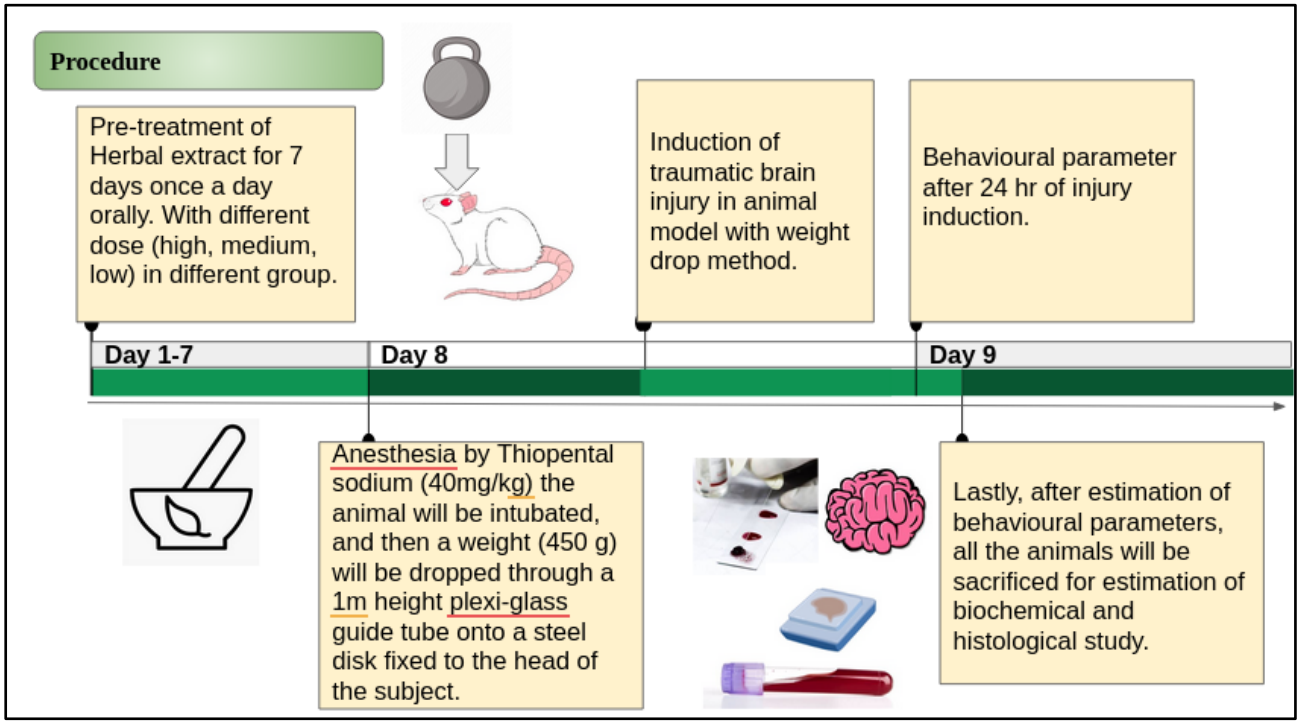


Fig 3.6.1: Experimental design of study

3.7 BEHAVIOURAL ASSESSMENT:

Behavioural training of animal were done before conducting experiment. Neurobehavioural parameters are performed to check cognitive behaviour of animal with help of actophotometer and rearing test whereas NADT was used to check memory deficits associated to traumatic brain injury. Locomotory and motor balance is a feature, mainly effected with trauma or neurological incision, to verify beam walk test, grip strength (Wire hanging test) and rotarod tests were performed.

3.7.1 Time to right:

Righting is reflex to form prone position when animal is placed on supine position. When an animal is subjected to trauma, a key clinical indicator of brain injury is the occurrence of unconsciousness. To evaluate this aspect, we measured the duration it took for an animal to regain consciousness after trauma induction using the marmaour weight drop method. The time measurement begins from the moment of anaesthesia administration and continues until the animal exhibits the righting reflex. (Fletcher and Mentis, 2017)(Berman et al., 2023)

3.7.2 Locomotory assessment:

1. Beam Walking Test:

Brain injury is known to have an impact on balance and motor coordination. Depending on the location and extent of the brain injury, disruptions in balance and limb functionality can be prominently observed. To evaluate the effects on locomotor activity, a beam walking test was conducted both 24 hours before and after the surgery. This involved taking an average of three readings with a 30-second interval. During the test, the animal was positioned at an initial marked point on a beam that measured 125 cm in length and 2.5 cm in width. The beam was elevated at a height of 70 cm. The recorded metric was the time it took for the animal to traverse the beam and reach its cage. It is notable that animals with injuries or impairments often exhibit prolonged crossing times, a tendency to grasp onto the beam, or even instances of falling, in contrast to the smoother performance of healthy animals.

(Ficiur, Faraji and Metz, 2016; Care and Instruments, 2001)

2. Grip strength test/ Wire hanging test:

The wire hanging test is utilized to gauge the animal's grasp strength subsequent to a brain injury. Post traumatic brain injury (TBI), the muscular strength was gauged by utilizing a 5 mm wire extended between two poles positioned at a height of 60 cm. The animals were positioned on the wire with their forelimbs, and they endeavored to ascend using their hind limbs. However, the weakening of muscle strength resulting from the injury often led to a loss of grip and subsequent falling. This experiment was carried out both before and 24 hours after inducing the injury, and the time it took for the animal to fall, known as the "latency to fall," was recorded.
(Fan et al., 2005; Naeem et al., 2019)

3. Rotarod:

The rotarod apparatus comprises a rotating rod with varying speeds of rotation. When on this rotating surface, animals encounter challenges in preserving their equilibrium. The animals underwent training to acclimate to this apparatus. Subsequently, the duration taken for the animal to lose balance was gauged under different speeds of rotation, both before and after the injury. Specifically, the time it took for the animal to fall at distinct or identical speeds was measured. The delay in falling, referred to as the "latency to fall," was observed at a rotational speed of 10 rpm both prior to the injury and 24 hours after it was induced.

4. Rearing test / cylinder test:

Rearing test is also called as cylinder test, that helps to evaluate the motor function of subjected animals. Rearing is observed, when rat tries to use support of any object with their forelimb. Here, rat will be placed in transparent cylinder, big enough to have 2 cm of extra space for animal to move. Cylinder height will be long enough that animal is unable to touch the upper end of cylinder by mouth. 1 point is given every time animal lift and touch wall of cylinder their left front limb, right front limb or both front limb simultaneously. (Magno et al., 2019).

3.7.3 Neurobehavioural parameter:

1. Actophotometer:

Actophotometer is instrument with built-in photo sensor and 4 digital counters of photoelectric cells that reads animal’s locomotion. This helps to measure total spontaneous activity. TBI is associated with poor motor activity that index alertness in animals brain. A continuous beam is present directing towards photocell, when animal moves and cut the beam a reading is generated.

Instrument can be used for both mice and rats. Animals were weighted and kept in cell of instrument one by one. Instrument is turned on and cape is closed. Reading was noted for 5 minutes for each animal. % decrease in motor activity was measured.

3.7.4 Cognitive Test

1. Novel Arm Discrimination Test (NADT):

NADT is a test performed using Y- maze to assess exploratory behaviour. An innate behaviour of rodents, it helps to estimate rat short-term memory, assess spatial working memory (Adeosun et al., 2014). Y- maze instrument consists of three arms, which are randomly tagged as the starting arm, novel or blocked arm, and another arm. The test is performed in 2 phases : Phase 1 as acquition phase and Phase 2 as retention phase. Each phase holds 5 min with interval of 1 hour. The test is initiated by permitting the rat to explore the arms of Y maze in acqition phase for 5 min where one arm is blocked. After one hour of interval, in retention phase of experiment animal is allowed to explore the Y maze for 5 minutes to all the arms freely without resisting any novel arm. During the test, if rat is not be able to identify the novel arm indicates alteration in spatial memory. Total time spent in novel arm is recorded. (Aldhahri et al., 2022)

3.7.5 Water content estimation in brain:

Water content was use to check brain edema in brain. Disruption and damage to BBB causes leukocyte infiltration following edema in brain leading to water accumulation. Water content was measured by sacrificing animal, isolating half brain of animal. Wet weight was noted before dipping brain for 30 minute in absolute alcohol. Later dry weight was measured after placing dipped sample in petridish and keeping in a hot air oven for 24 hr at 55±5 °C temperature.

The value was placed in given formula to find % brain water content:

% BWC= [(wet weight of brain – dry weight of brain)/ wet weight of brain] × 100. The value is expressed at % BWC / 10 gm of animal body weight (Sebastiani et al., 2017).

3.7.8 Biochemical estimation:

1. Lipid Peroxidation estimation (MDA levels):

Lipid peroxidation term used to refer breakdown of oxidative. Generating free radicals captures electrons from lipid molecules present in cell membrane leading to cell damage. In process of lipid peroxidation. Malondialdehyde is obtained as final product that plays crucial role as marker. For

LPO estimation 100 mg of brain cortex was homogenized and supernatant was collected. Remaining homogenate was discarded. Centrifugation was done at 3000 rpm for 10 minutes at temperature 25ºC. Furthermore 1.5 ml thiobarbituric acid (TBA), 1.5 ml acetic acid, 0.2 ml sodium dodecyl sulfate (SDS) and 0.7 ml of MilliQ water were mixed in cell pellet tubes whereas for control tubes 0.1 ml of HBSS was added. Test tubes were placed in test tube holder and kept in water bath for 1 hr at 95°C temperature. Tubes were boiled, and then cooled for 1 minute before adding 1 milliQ water to it. Lastly, 5 ml butanol: pyridine (15:1) was added to each tube and were vortexed for about 5 minutes to achieve proper mix. The organic layer than centrifuged at 3000 rpm for 10 minutes. The absorbance was checked at 532 nm to estimate level of MDA. The concentration of MDA was determined using TEP- prepared standard curve in µM/mg of brain tissue(Ohkawa, Ohishi and Yagi, 1979; Dash and Chavali, 2018).

2. Estimation of Nitrite Levels:

Nitric oxide (NO) level shows significant level of oxidative stress. Estimation of NO was performed according to (Sehba et al., 2000) with little modification. 100 mg of brain cortex was homogenized in 4 ml of 0.2 M ice-cold phosphate buffer pH: 7.6. Griess reagent used was made
with same amount of each chemical namely, 1% napthylethylenediamine hydrochloric acid, 1% sulphanilamide, and 5% phosphoric acid. The chemicals were made to dissolve in PBS. Sample tubes were prepared by adding 300 µl of homogenated sample + 100 µl of griess reagent to 2.6 ml PBS. For blank reading homogenized sample was absent. The sample tubes were incubated for 30 minutes at room temperature. Absorbance reading was recorded at 548 nm (Liy, Puzi and Jose, 2021).

3. Estimation of reduced glutathione:

Reduced form of glutathione (GSH) is an anti oxidative marker. Decrease in GSH shows the oxidative damage. For brain GSH (anti oxidativeenzyme) estimation method was performed as earlier method with mild modification (Khan et al., 2012). Briefly 100 mg of brain coretex was homogenized in 5 ml 0.2 M Iced- PBS with pH 7.6. The sample tube containing homogenate received 100 µl of 25% trichloroacetic acid. Tubes were centrifuged for 10 mintues at 25ºC at 5000 rpm speed. For sample, 1 ml of PBS, supernatant and DTNB were mixed and vortexed and incubated for 5 minutes. For reference 2 ml of PBS and 1 ml DTNB was mixed. The absorbance was measured at 412 nm in UV spectrophotometer. Calculation was done using standard curve of GSH (Liy,Puzi and Jose, 2021).

3.7.9 Histopathology study:

After being separated, the brain tissues were preserved in 10 % formalin solution for histological analysis and cleaned in an ice-cold 0.9 percent saline solution. For histopathology, hematoxyline and eosin stains were employed. We ran some tap water over the fixative to clean it. After that, a paraffin block-sized piece of brain tissue was cut out after being dissected. Following the numbering process, 95 % alcohol was used twice throughout the dehydration process. Following that, it was cleaned for five to ten minutes using three variations of xylene. Alcohol and xylene were utilized to carry out the hydration and deparaffinization processes. The hematoxyline and eosin staining was then completed. To mount each component, dibutylphthalate polystyrene xylene (DPX) mountant was utilized. The sections were examined under a light microscope.

3.7.10 Statistical analysis:

The statistical analysis, was performed by using Prism 9.1 software. Data was analysed by One way ANOVA with Tukey multiple comparison test as post hoc test. The bar graph represents mean ± SEM, with n=6. The statistical significance value considered was p value = <0.05

Chapter-4

Results

CHAPTER 4: RESULTS

4.1: Extraction result:

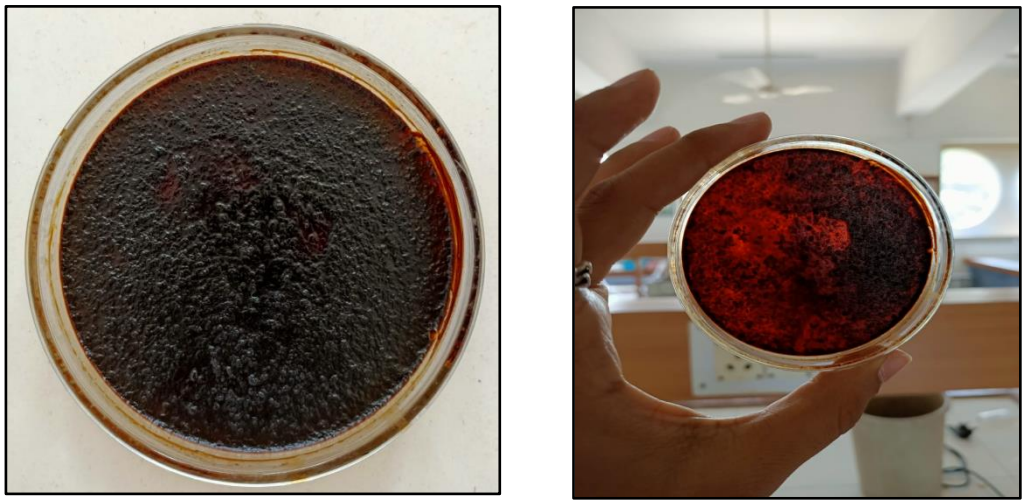


Fig: 4.1.1: The pictorial diagram illustrates *Nardostachys Jatamansi* extract obtained from soxhlet extraction method using ethanol as menstrumm.

Table: 4.1.1: Extraction yield

Dried root powder	**Extraction**	**Solvent**	**Expected yeild**	**Practical yeild**
10g	soxhlet extraction	100ml- 90% ethanol	8 %	6.18 %

4.2 Ameliorated effect of ethanolic extract of *Nardostachys Jatamansi* (NJ) on TTR of TBI exposed rats:

Righting response is an innate reflex wherein an animal spontaneously returns to a standing position from a supine or lateral posture. TTR explains clinical characteristics of unconsciousness during primary state of TBI. It is an amount of time taken by the animal to gain consciousness after experimental TBI induced unconsciousness. To understand the interference of anaesthesia, normal control animals are simultaneously observed. TTR is calculated from animal being anaesthetized, exposed to trauma to time being conscious on it's feet. Fig 4.1.1,

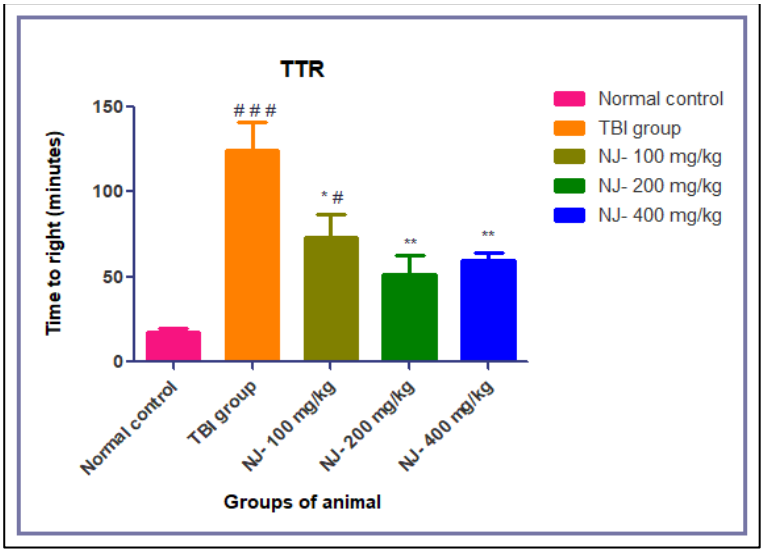


Figure 4.2.1: Effect of *Nardostachys Jatamansi* at 100mg/kg, 200mg/kg and 400mg/kg on Time To Right (TTR) in experimental model of Traumatic brain injury.All the values are expressed in mean ± SEM (n=6). #p<0.05, ##p<0.01, ###p<0.001 compared to normal control, *p<0.05, **p<0.01 compared to TBI group, using one-way ANOVA (Tukey-multiple comparisons test).

Fig 4.2.1 shows that time taken by control group was approx. 25 minutes while TBI group exhibits significant increase in TTR upto 130 minutes. When compared with 100 mg/kg- NJ group of animals, time taken was almost similar to disease control showing no significant difference.

Whereas animals pretreated with NJ with 200 mg/k and 400 mg/kg dose shows comparatively significant improvement. That is, animal treated with 200 and 400 mg/kg dose of NJ took around 55- 60 minutes for righting reflex.

4.3 Ethanolic extract of *Nardostachys Jatamansi* enhances locomotory activity, assessed by beam walking test.

Beam walking test was used to inspect impact of weight dropped on motor coordination. Beam walking test is marked as time taken by animal to cross the beam from unfamiliar position to the home cage. The test was performed after 24 hours of TBI induction. Study shows significant improvement in ability to cross the beam swiftly in NJ treated animal at different doses (100 mg/kg, 200 mg/kg, 400 mg/kg) than TBI induced animal group. The time taken by normal control and TBI group of animal are 5 seconds and 15 seconds showcasing worsening in TBI group.

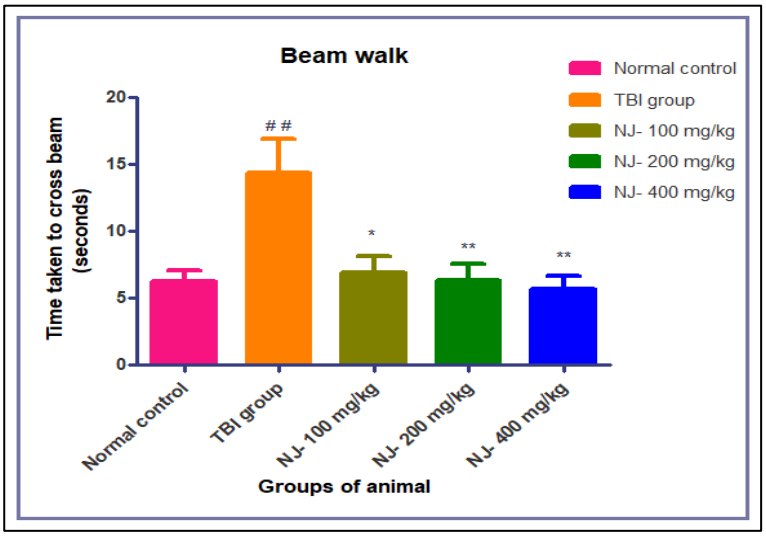


Figure 4.3.1: Effect of *Nardostachys Jatamansi* at 100 mg/kg, 200 mg/kg and 400 mg/kg on beam walking test for motor coordination in experimental model of traumatic brain injury. All the values expressed in mean ± SEM (n=6). #p<0.05, ##p<0.01 compared to normal control, *p<0.05, **p<0.01 compared to TBI group, using one-way ANOVA (Tukey-multiple comparisons test).

The given diagram illustrates time taken to cross beam by different groups of animals (Normal Control, TBI group, NJ 100, NJ 200, NJ 400 (mg/kg)

According to the data, group of normal control animals tends to take around 7 seconds or lesser time to pass the test, while the animal infected of the disease group use to take approximately 17 seconds, meanwhile TBI animals with treatment of NJ (100 mg/kg, 200 mg/kg, 400 mg/kg) of dosage takes 8, 7, 6 seconds respectively. In addition to this, animal with TBI were seen to slip while crossing the beam. Hence, showing better outcomes of drug.

4.4 Ethanolic extract of *Nardostachys Jatamansi* enhances locomotory activity, assessed by wire grip strength.

Wire grip strength is test performed to check ability of animal to withstand grip by forearms and ability to hold its weight. Animal severely affected by TBI may not grip the wire for longer time.

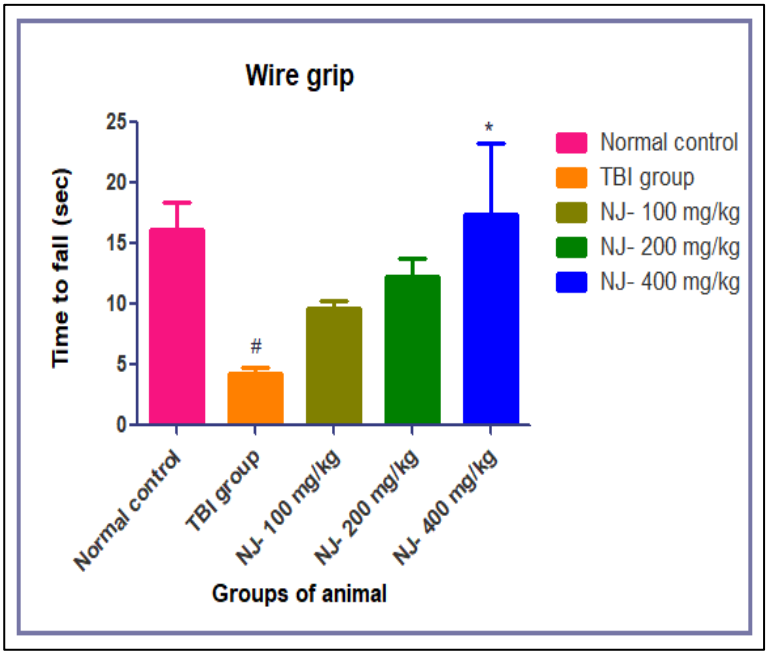


Figure 4.4.1: Effect of *Nardostachys Jatamansi* at 100 mg/kg, 200 mg/kg and 400 mg/kg on wire grip strength for motor coordination in experimental model of traumatic brain injury. All the values are expressed in mean±SEM (n=6). #p<0.05 compared to normal control, *p<0.05 compared to TBI group, using one-way ANOVA (Tukey-multiple comparisons test).

The graph represents disability of animal exposed to TBI as shown, the animal was not able to hold wire more than 5 second whereas normal control animal were able to hang on wire for almost 15 seconds, whereas in treatment group, the significant result was obtained in highest dose i.e 400 mg/kg rather than low and mid dose treatment group. Hence NJ was prominently effective in 400 mg/kg treated group as compare to rest.

4.5 Ethanolic extract of *Nardostachys Jatamansi* enhances locomotory activity, assessed by rotarod.

Rotarod is simplest yet easily observable physical parameter to evaluate effectiveness of treatment, as it evaluates motor functioning as well as it's coordination.

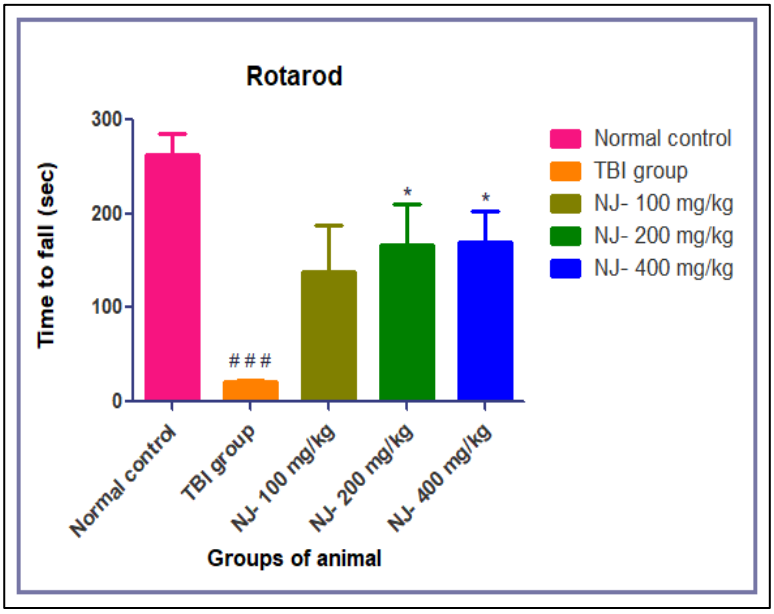


Figure 4.5.1: Effect of *Nardostachys Jatamansi* at 100 mg/kg, 200 mg/kg and 400 mg/kg on rotarod for motor coordination in experimental model of traumatic brain injury. All the values are expressed in mean±SEM (n=6). ###p<0.001 compared to normal control,*p<0.05 compared to TBI group, using one-way ANOVA (Tukey multiple comparisons test)

The presented bar chart illustrates a rapid decline in the performance of animals on the rotarod apparatus within the Traumatic Brain Injury (TBI) cohort in comparison to the control group. While the control group animals exhibit a substantial walking duration of nearly 300 seconds, the 100 mg/kg treatment subgroup within the TBI group does not display a statistically significant distinction. However, notable enhancement is discernible in animals treated with NJ at doses of 200 mg/kg and 400 mg/kg.

4.6 Ethanolic extract of NJ mitigates the motor function deficits in TBI- exposed rats in rearing test:

Figure 4.6 shows rearing activity of rat exposed to TBI and statistical significance improvement in group pre-treated with NJ for 7 days prior to TBI. The number of rearing, is count when rat stands as their natural habit for exploration. The repeated up and down behaviour of rat is observed.

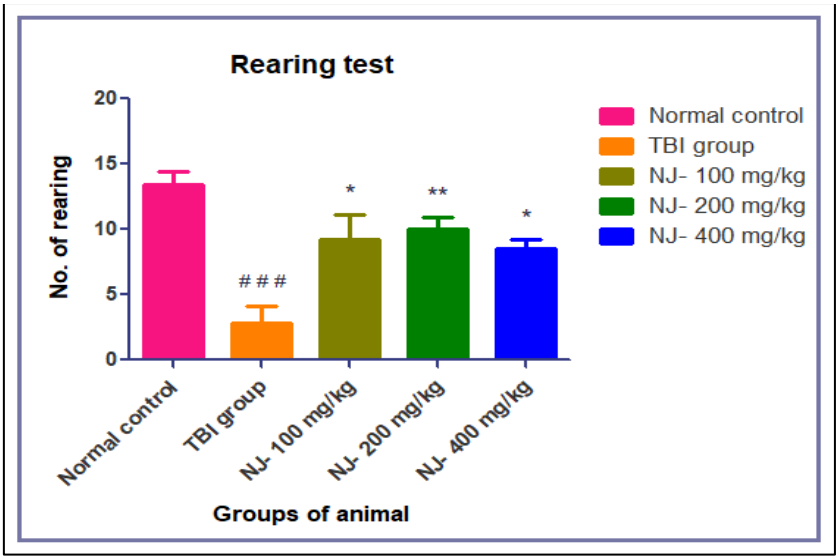


Figure 4.6.1: Effect of 7 days oral NJ pre treatment on rearing test in an experimental rat model of TBI. Each bar represents the mean ± SEM (n=6). ###p<0.001 compared to normal control, *p<0.05, **p<0.01 compared to TBI group, using one-way ANOVA (Tukey multiple comparisons test).

According to the graphs shown, significant improvement in rearing behaviour in treated group with all the doses of NJ as compared to traumatized rats. While the cohorts administered with doses of 100 mg/kg and 400 mg/kg exhibit reduced statistical significance when contrasted with the group subjected to a 200 mg/kg dosage of NJ.

4.7 Ethanolic extract of NJ alleviates the attenuated learning memory of TBI-exposed rats in Novel Arm Discrimination Test (NADT):

Novel arm discrimination test is to check cognitive ability of animal when introduced to trauma and evaluate protection of drug against alteration in cognition after brain injury. The time spend by animal in blocked/novel arm in acquisition phase is evaluated.

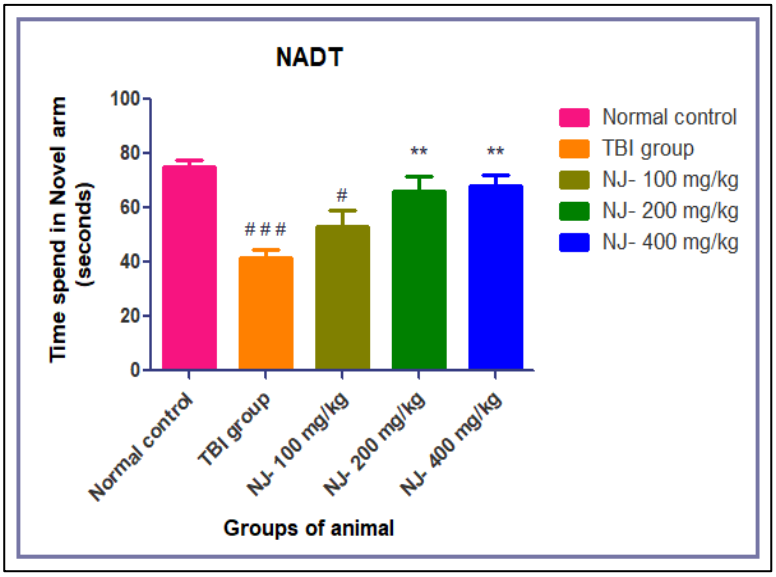


Figure 4.7.1 Effect of ethanolic extract of NJ with 100 mg/kg, 200 mg/kg and 400 mg/kg dose treatment in novel arm discrimination test in experimental rat model of TBI. Each bar represents the mean ± SEM (n=6). #p<0.05, ###p<0.001 compared to normal control **p<0.01 compared to TBI group, using one-way ANOVA (Tukey-multiple comparisons test).

The result shows limited amount of time spend in Novel arm by TBI exposed rats as compare to rats of normal control group. However treatment groups shows elevation in time spend in novel arm almost equivalent to normal control animal. Though no significant difference was observed in NJ-100 mg/kg animal group, while significant difference in other two treated groups were observed when compared with disease control animals.

4.8 NJ evidently reduced the level of Malondialdehyde (MDA) in TBI- disease control group of animal.

The MDA is hallmark for lipid peroxidase (LPO). The estimation of level of LPO in brain tissue access oxidative damage followed by TBI. The bar graph 4.8.1 represents the level of MDA concentration in 100 mg of brain tissue and concentration in expressed in M/ 100 mg of brain tissue.

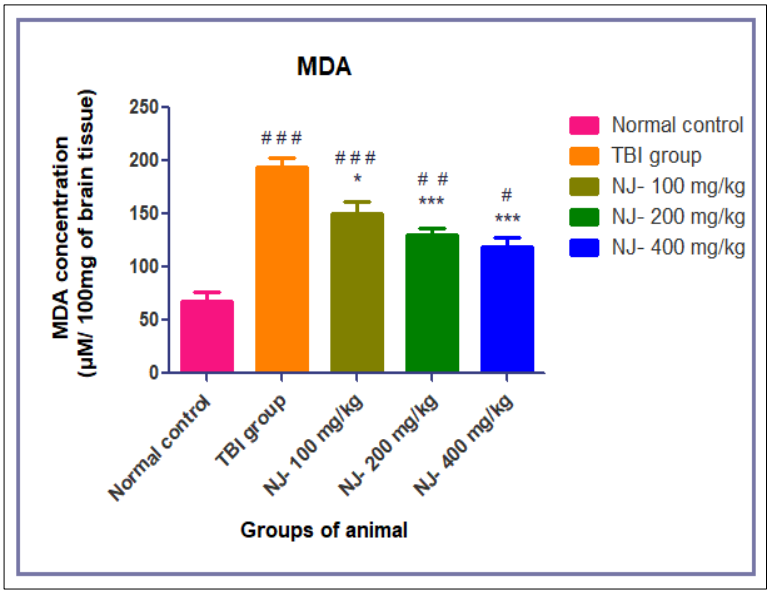


Figure 4.8.1 Effect of ethanolic extract of NJ with 100 mg/kg, 200 mg/kg and 400 mg/kg dose treatment against lipid peroxidations in experimental rat model of TBI.Each bar represents the mean ± SEM (n=6). #p<0.05, ##p<0.01, ###p< 0.001 compared to normal control, *p<0.05, ***p<0.001 compared to TBI group, using one-way ANOVA (Tukey multiple comparisons test).

As graphs shows level of MDA in TBI exposed animal is hiked significantly as compare to normal control group. Increased MDA level significance peroxidation process in tissue. The concentration later with treatment of NJ is significantly decreased with increase in dose when compared to disease group. Though the disease +treated group do not show similar result to normal control, but shows improvisation when compared with disease exposed group. Hence result shows protective effect of NJ against peroxidation process occurs post TBI.

4.9 NJ administration significantly lowers nitrite levels in TBI exposed animal.

The figure 4.9.1 represents the analysis of nitrite levels in brain tissue to explore the potential impact on the brain following traumatic brain injury. The x-axis of the figure likely represents the different experimental groups, such as the normal control, TBI-induced group, and the three treatment groups (NJ- 100 mg/kg, NJ- 200 mg/kg, NJ- 400 mg/kg). The y-axis represents the concentration or level of nitrite in the brain tissue.

Analysis with the one-way ANOVA followed by Tukey multiple comparison test demonstrated the changes in nitrite levels in the different experimental groups. It shows that the TBI- induced group exhibits the highest level of nitrite, indicating an elevated production of nitric oxide (NO) following the injury. This finding supports the notion that NO plays a significant role as a damaging factor in TBI by triggering the oxidative stress.

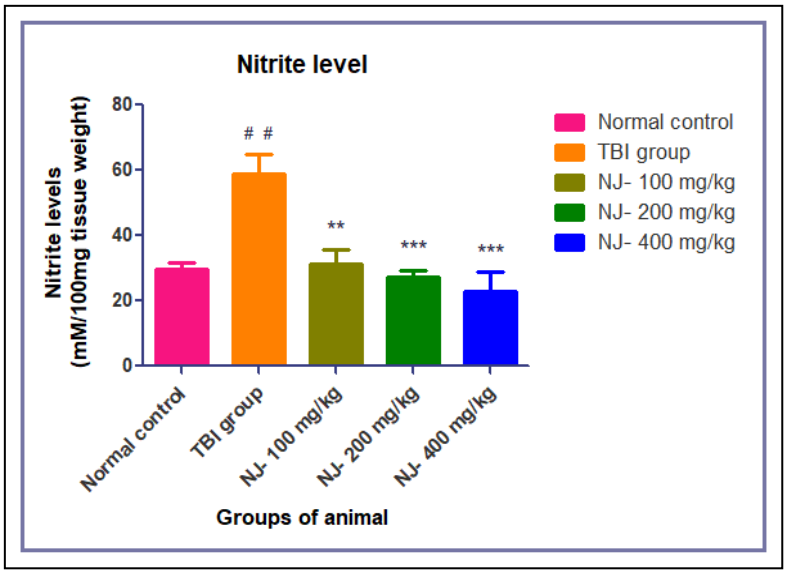


Figure 4.9.1 Effect of ethanolic extract of NJ in nitrite level in 100 mg of brain tissue against experiment model of TBI. Each bar represents the mean ± SEM (n=6). ##p<0.01 compared to normal control, **p<0.01,***p<0.001 compared to TBI group, using one-way ANOVA (Tukey multiple comparisons test).

The concentration of nitrite in TBI exposed group is significantly highest as compare to other groups. The treatment group showed significant difference trend by reducing the level of nitrite with all theses of NJ as compared to TBI induced group. The effectiveness of NJ in reducing nitrite level in brain in TBI exposed rats are in dose dependent manner. Thus results provides mitigating action of NJ in oxidative damage in TBI.

4.10 NJ remarkably elevate level of reduced glutathione (GSH) in animal group with TBI.

Figure 4.10.1 shows different level of GSH in various animal group Reduced glutathione is an antioxidant enzyme. It function is to provide protection against oxidative stress-induced damage. Decreased level of GSH in brain indicates oxidative stress. To evaluate the effectiveness of NJ on GSH level in TBI.

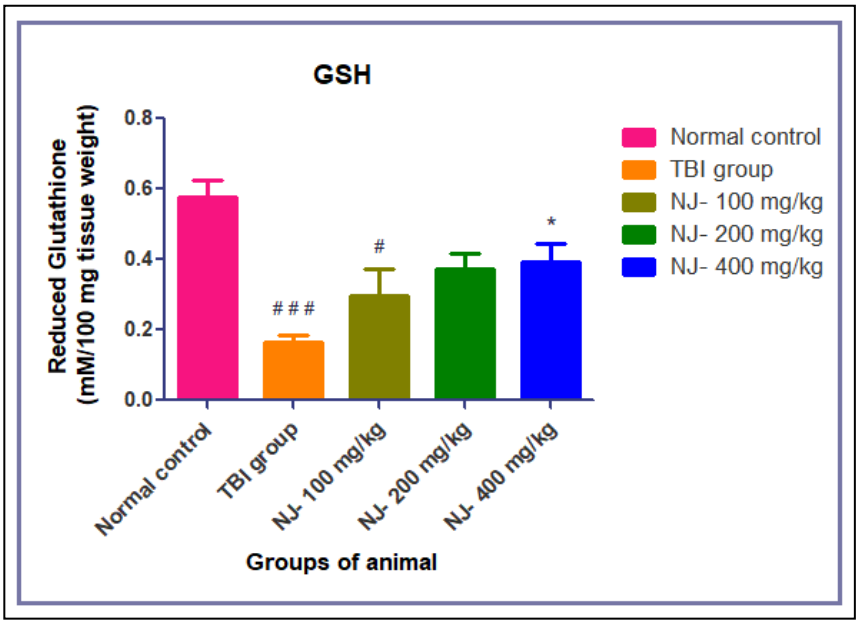


Figure 4.10.1: Effect of *Nardostachys Jatamansi* on GSH level in 100 mg of brain tissue sample in experimental rat model of TBI. Each bar represents the mean ± SEM (n=6)., #p<0.05, ###p<0.001 compared to normal control,*p<0.05 compared to TBI group using one-way ANOVA (Tukey multiple comparisons test).

The study of different doses of NJ in different groups of animals, indicates decrease level of reduced glutathione in TBI exposed animals as compared to normal control. Thus indicates TBI induced oxidative damage to the brain. In NJ administered groups though slight visible increase in GSH level is seen in groups treated with 100 mg/kg and 200 mg/kg dose, but no significant difference was observed in level of GSH as compared to disease control group suggesting moderate effect in lower and mid-dose of NJ to elevate GSH level. However, group treated with 400 mg/kg dose of NJ shows significant increase in GSH level as compare to disease control group. Hence, this analysis provides strong improvement against level of oxidative stress in the highest dose of NJ.

4.11: Histopathology analysis:

The figure 4.11.1 depicts histopathological analysis of animal brain tissue of various group. The cortex region of rat's brain was investigated. The picture represents normal control, disease (TBI) exposed group, NJ- 100mg/kg, NJ- 200 mg/kg, NJ- 400 mg/kg treated groups. The brain tissue was stained using Hematoxyline & eosin (H&E) dye.

Vasogenic edema is result of fluid accumulation in extracellular space caused by BBB disruption, the histology shows normal characteristic of brain tissue in normal control group of animal. The disease control group exposed to TBI exhibits vasogenic edema. Whereas remarkable reduction in amount of vasogenic edema was found in treated group. Though NJ administered 100 mg/kg dose shows minor presence of vasogenic edema, but 200 mg/kg dose shows slight perivascular edema and improvement in vasogenic edema as compare to disease control and 100 mg/kg dosed group animal. The group with the highest dose i.e. shows 400 mg/g shows the best histological features similar to normal control group.

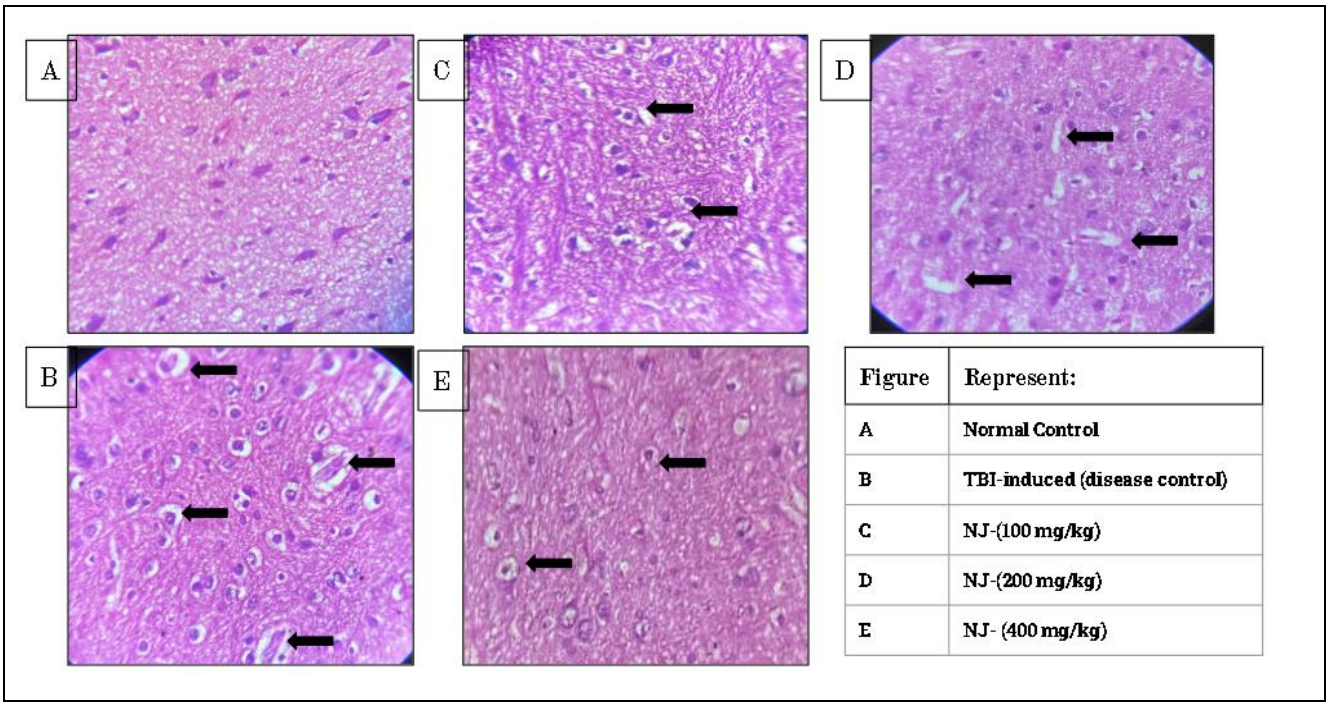


Figure	Represent:
A	Normal Control
B	TBI-induced (disease control)
C	NJ-(100 mg/kg)
D	NJ-(200 mg/kg)
E	NJ- (400 mg/kg)

Fig. 4.11.1 Representative photographs of cortex region of brain after 24 h of TBI induced rats. 15 days pretreatment of NJ 250 mg/kg b.w., respectively. Each section was stained with haematoxylin and eosin. Magnification 45x

Chapter-5

Discussion

CHAPTER 5: DISCUSSION

TBI can be described as neurological condition caused by mechanical force on head leading to disruption in normal brain function and structure, ending to temporary or permanent disability in normal life style. TBI today is major cause to mortality and morbidity. Pathophysiology of TBI can be labelled in 2 stages: primary state and secondary state. Primary stage is time injury occurs. That lead to long term impact on brain caused by several mechanism like excitotoxicity, ionic disturbance, cytokine burst, oxidative stress and neuroinflammation. This state is known as secondary injury. The primary injury is irreversible and hence targeting secondary injury pathways is crucial to prevent further damage to brain. The injury can also be classified as focal injury or diffuse injury. Diffuse injury is widespread injury rather than a local or focused injury. To confirm the level and presence of TBI in emergency cases, computerised scans are conducted. The diffused injury is critical to confirm and hence, to understand underlying pathways, research on diffusional injury should be appreciated. To imitate close head diffusional injury, we performed marmarou's weight-drop method to induce TBI in rats. Injury disoriented locomotor as well as ability of rats exposed to TBI examined by increased in time taken to perform beam walk test, time to fall in rotarod, time spend in closed arm, time spent in novel arm in Y-maze and actophotometer count. All the test helped to provide cognitive and behaviour deficits post TBI. The pretreatment of ethanolic extract of NJ in TBI for 7 days displays significant improvement in locomotory and cognitive behaviour of animal in compare to disease induced group. The mechanism of action of NJ is yet to establish but many studies shows generous results to postulate neuroprotective effect of NJ (Prabhu, Sudhakar Karanth and Rao, 1994) Examined drug against function of gamma amino butyric acid (GABA) in ischaemic stroke providing enhancement in it's function and lowering glutamargic spurge leading to excitotoxicity.

Disruption in blood brain barrier (BBB) plays important role in development of secondary mechanism post TBI. In the study to evaluate loosening of BBB water content was measured. As BBB disruption give rise to leakage and permeability of water content in brain. Study postulated augmented level of water in disease control as compare to normal rat brain, providing support to brain edema formation and increase in intracranial pressure worsening critical TBI condition (Carney et al., 2017; Dash and Chavali, 2018). Where % water content of TBI induced group was high as compared to normal control. In treatment group dose dependent declination was observed respectively. In TBI pathogenesis due to BBB disruption, influx of Ca^{+2} and Na^{+2} and fluid

accumulation can be seen as a result of surge in excitatory neurotransmitters namely glutamate accumulation further activating receptors like N-methyl-D-aspartate (NMDA) or 2- amino-3-hydroxy-5-isoxazolepropionate (AMPA)/kainate (Erdin and Wegmann, 1996). Glutamate also interfere with generation of oxidative stress in degenerative disorders. Oxidative stress plays important pathological role in TBI worsening due to increase in oxygen level demand caused by primary injury. To mitigate oxidative stress, free radical scavenging antioxidants are present in body, but shortage of such antioxidant enzymes like superoxide dismutase, ascorbate and reduced form of glutathione escalate oxidative damage to brain. The mechanism of GSH may lay on inter or intra cellular signal transmission. Thus, drop in GSH level in brain may effect behavioural and cognitive function. The prime reason for GSH loss is glutathione mixed disulphides (PrSSG) protein generation and mislaying of thiol protein (DHARMAWAN, 1990).However, from groups treated with NJ at different dose the group received highest dose (NJ-400 mg/kg) shows statistical significant hold on maintaining higher GSH level as compared to disease exposed and other dose treated groups.

Lipid peroxidation is another contributing factor playing role in oxidative stress generation that alters normal signalling pathways of membrane. Molondialdehye (MDA) is secondary byproduct of the peroxidation. That can work as hallmark to analyse concentration of MDA in the brain. (Ohkawa, Ohishi and Yagi, 1979; Pearn et al., 2017) In the study TBI exposed animals shows higher level of MDA concentration as compared to normal control animal. Whereas NJ treated group shows prominent decrease in level of MDA concentration providing protective outcome against lipid peroxidation. Nitrite is another oxidative stress modular where nitrite is converted to nitric oxide (NO) by reduction during pathological environment. NO further interact with superoxide free radicals forming reactive nitrogen species which is super toxic agent for neuronal injuries (Wada et al., 1998). The level of nitrites in trauma induced animals was found to be extremely high in compare to normal animals. While, down fall of concentration on nitrite was observed respective to the doses.

In the study of histopathology of brains of rats, performed using H&E stain. Where the cortex region was taken under consideration. The microscope was adjusted to 45x magnification and histoslides were observed. Where 4 different types of edema were observed: Vasogenic edema, interstitial, osmotic and cellular edema. Vasogenic edema is promptly observed in TBI like condition. Vasogenic edema can be termed as fluid accumulation in brain cell post injury where BBB disruption is observed whereas cellular toxic is independent to BBB damage and solely caused

by swollen cells (Jha, 2003). In our findings, number of vasogenic edema was higher in disease manifested group as compare to normal control brain histology. In NJ- 100 mg/kg treatment there was improvement in vasogenic edema as compare to diseased group brain but cellular edema was present with no significant difference between diseased group. Meanwhile 200 mg/kg and 400 mg/kg treated group shows better prevention from fuild accumulation in brain histology study as compared to other groups.

In brief, the collected data illustrates that administering ethanolic extract of NJ as a pretreatment for 7 days offers protection against traumatic brain injury (TBI) in various aspects, including the behaviour and cognitive performance of the animals, as well as biochemical and histological changes observed in the TBI-exposed groups with pretreatment. This protective effect appears to primarily involve enhancements in reduced glutathione levels, reductions in peroxidation and nitrite levels in the brain following TBI, ultimately leading to an overall safeguarding of neural tissue primarily through the inhibition of oxidative stress. Consequently, the study implies that NJ possesses antioxidative properties effective against oxidative stress resulting from TBI. Consequently, NJ could potentially serve as a promising candidate for preventive therapeutic interventions for traumatic brain injury (Hunter, Mackay and Rogers, 1998; Sinha, Chaudhary and Kumar Gupta, 2002).

Chapter-6

Conclusion

CHAPTER 6: CONCLUSION

In conclusion, this study investigated the preventive effects of *Nardostachys Jatamansi* (NJ) at different doses (100 mg/kg, 200 mg/kg, and 400 mg/kg) against experimental traumatic brain injury (TBI) induced by the Marmarou's weight drop method in rats. The results revealed that NJ exhibited neuroprotective properties by reducing oxidative stress levels, thereby preserving locomotors activity and cognition in TBI-exposed animals pretreated with NJ. The most significant outcome was observed in the group treated with 400 mg/kg of NJ, while the 200 mg/kg dose also showed improvements in mitigating TBI pathogenesis. These findings confirm our hypothesis that the ethanolic extract of *Nardostachys Jatamansi* possesses antioxidant properties, contributing to its neuroprotective effects. However, further investigations are necessary to understand the underlying working pathways and identify the specific chemical constituents responsible for these observed effects. This additional research will provide a more comprehensive understanding of the mechanisms involved and enhance the potential applications of *Nardostachys Jatamansi* as a therapeutic agent for TBI.

Chapter-7

Reference

CHAPTER 7: REFERENCES

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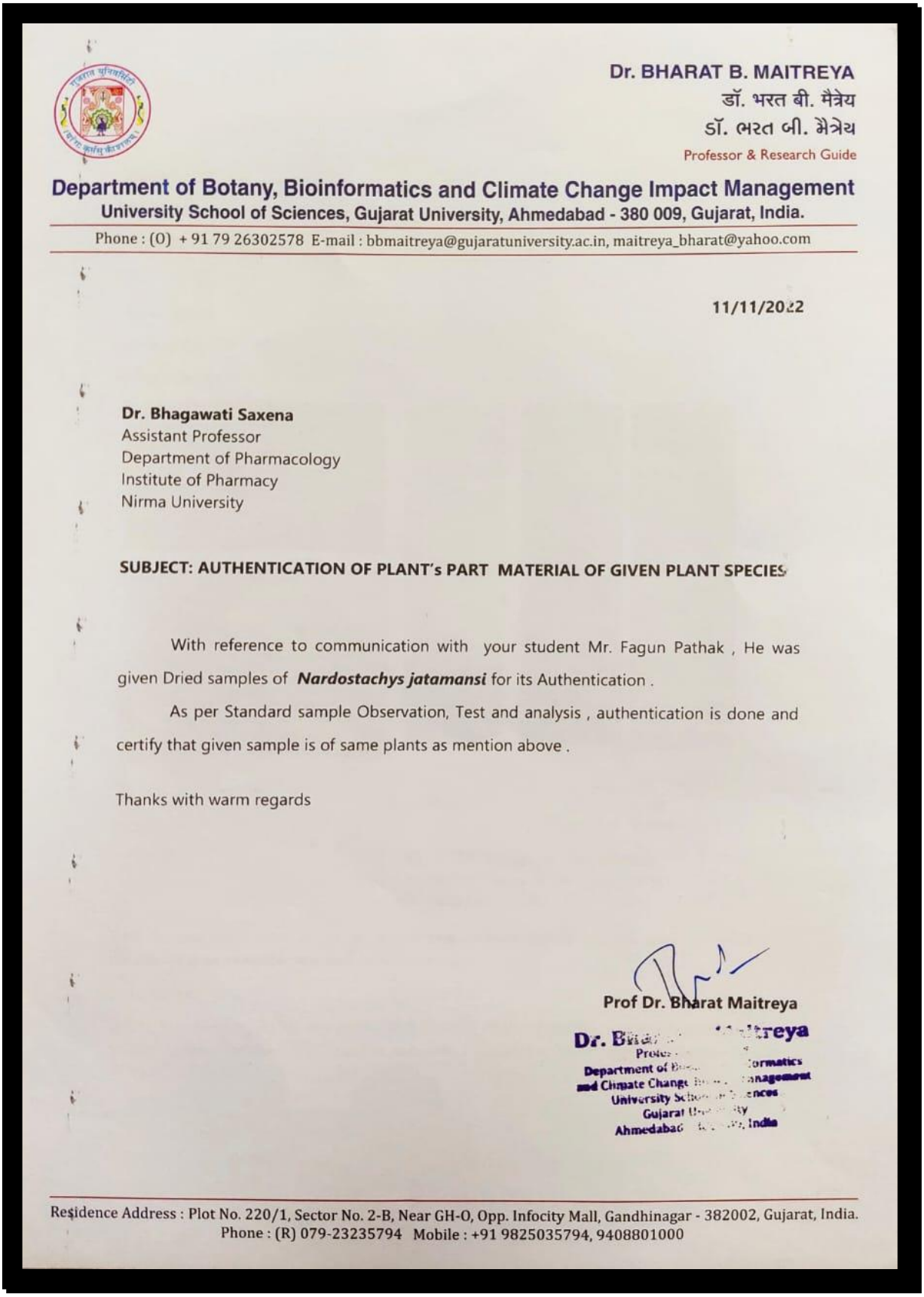
Certificate



Dr. BHARAT B. MAITREYA
डॉ. भरत बी. मैत्रेय
ડૉ. ભરત બી. મૈત્રેય
Professor & Research Guide

Department of Botany, Bioinformatics and Climate Change Impact Management
University School of Sciences, Gujarat University, Ahmedabad - 380 009, Gujarat, India.
Phone : (O) + 91 79 26302578 E-mail : bbmaitreya@gujaratuniversity.ac.in, maitreya_bharat@yahoo.com

11/11/2022

Dr. Bhagawati Saxena
Assistant Professor
Department of Pharmacology
Institute of Pharmacy
Nirma University

SUBJECT: AUTHENTICATION OF PLANT's PART MATERIAL OF GIVEN PLANT SPECIES

With reference to communication with your student Mr. Fagun Pathak , He was given Dried samples of ***Nardostachys jatamansi*** for its Authentication .

As per Standard sample Observation, Test and analysis , authentication is done and certify that given sample is of same plants as mention above .

Thanks with warm regards

Prof Dr. Bharat Maitreya

Dr. B... ...treya
Profe...
Department of B... ...ormatics
and Climate Change ... ...anagement
University Sch... ...ences
Gujarat U... ...ity
Ahmedabad ... India

Residence Address : Plot No. 220/1, Sector No. 2-B, Near GH-O, Opp. Infocity Mall, Gandhinagar - 382002, Gujarat, India.
Phone : (R) 079-23235794 Mobile : +91 9825035794, 9408801000

Certificate

Sample: 2

Plant Name: *Nardostachys Jatamansi* L.

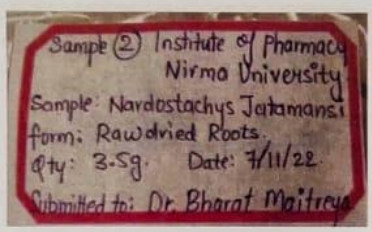
Organoleptic Characters:

Taste: woody

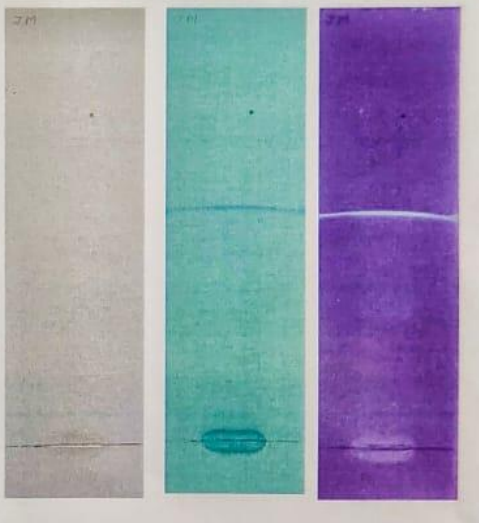
Odour: rancid

Texture: Smooth

Colour: Brown



Powder of Sample



Visible Light | UV 254 nm (Short Wavelength) | UV 356 nm (Long Wavelength)

Fig. 1 TLC Images of *Nardostachys Jatamansi* Leaves in Methanol Extract
Rf Value: 0.61, 0.72, 0.98

Here we compare the powder (Organoleptic) characteristic and TLC profile of given sample with the data of standard paper and their references.

References:

Jha, S. V., Bhagwat, A. M., & Pandita, N. S. (2012). Pharmacognostic and Phytochemical studies on the rhizome of Nardostachys jatamansi DC. using different extracts. *Pharmacognosy Journal*, *4*(33), 16-22

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