EFFECT OF *Pipernigrum* EXTRACT ON AN EXPERIMENTAL MODEL OF NEUROLOGICAL DISORDER

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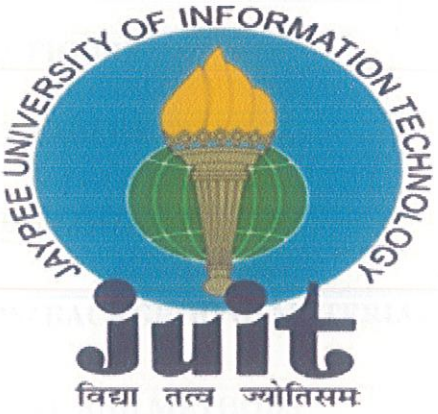

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CERTIFICATE

This is to certify that the work titled "EFFECT OF PIPER NIGRUM EXTRACT ON AN EXPERIMENTAL MODEL OF NEUROLOGICAL DISORDER " submitted by **Tanu Sharma** (081760) and **Priya Singh** (081777) in complete fulfillment for the award of degree of **B.Pharm**. of Jaypee University of Information and Technology, Waknaghat has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of Supervisor .....

Name of Supervisor Dr. Udaybanu

Designation Lecturer

Date

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SUMMARY

Cerebral ischemia is one of the major neurological disorder . The effect of piperine was studied in cerebral ischemic model of mice. The animals were divided into 4 groups (gp1-control, gp2-sham operated, gp3-cerebral ischemia, gp4-cerebral ischemia+ piperine,each group contain three mice.Then we performed various behavioural tests - Actophotometer test, Passive avoidance through task and beam walking test to study the effect of piperine . The piperine attenuated the ischemic disorder in mice induced with cerebral ischemia.

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Chapter 1

Introduction

Introduction

According to the recent studies stroke is one of the leading causes of mortality and morbidity worldwide. Approximately 20 million people each year will suffer from stroke and of these 5 million will not survive. (Dalal et al 2007) Developing countries account for 85% of global deaths from stroke (Gupta et al 2008). Effective screening, evaluation, and management strategies for stroke are well established in high-income countries, (Bath and Lees 2000) but these strategies have not been fully implemented in India. (Pandian 2007).

The evidence of the management and treatment of stroke in India is confined to small studies,but these highlight issues of lack of access, facilities and provision and lack of knowledge of stroke symptoms in the Indian population.

Cerebral ischemia cause neuronal damage by activation of series of neurotoxic events such as lipid peroxidation, free radical generation, activation of proteolytic enzymes and pathological gene activation leading to the formation of zone of infarction in the area where blood supply has been interrupted. As Piperine is having an antiinflammatory effect,it has been demonstrated in vitro experiments having an ability to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and hydroxyl radicals. Black pepper or piperine treatment has also been evidenced to lower lipid peroxidation in vivo and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress. Piperine has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals by this very property.

We carried out this work because our main area of interest is in pharmacology. As there is limited treatment and studies conducted on cerebral ischemia so we chosen this particular neurological disorder. Moreover, the basic etiology involved in cerebral ischemia is related to activation of free radical generation , lipid peroxidation and piperine is having an ablity to protect against oxidative damage by inhibiting free radicals and also lowers lipid peroxidation. The objective of present project is to study effect of piperine on cerebral ischemia by observing different behavioural studies.

The emphasis is laid on actophotometer test, beam walking test and passive avoidance step through task.

In this project we were mainly concerned with studying the effect of piperine extracted from piper nigrum on experimental model of neurological disorder. Amongst different kinds of neurological disorder we have chosen cerebral ischemia.We have induced cerebral ischemia in a swiss albino mice and segregated mice into three groups :

1) Group 1- control vehicle treated mice
2) Group 2- sham operated mice
3) Group 3- surgery induced mice + ischemic reperfusion
4) Group 4- surgery induced mice + ischemic reperfusion + piperine

Then compared the behavioural aspects of normal mice, mice induced with cerebral ischemia and mice induced with cerebral ischemia + piperine

Chapter 2

Review of Literature

Review of Literature

Cerebrovascular disease (CVD) is commonly and frequently encountered, endangering the people's life and work because of its high mortality and serious disability. Cerebral ischemia accounts for seventy percents of CVD. Each year in the United States approximately 700,000 individuals are afflicted with a stroke and currently there are almost 2 million survivors of stroke living in the US with prolonged disability. In China 1.5 million people die from stroke each year and in developed nations stroke is the third leading cause of death, only surpassed by heart disease and cancer. There are very few treatments for stroke and the development of new therapeutics is imperative. Cerebral ischemia may result from a variety of causes that impair cerebral blood flow (CBF) and lead to deprivation of both oxygen and glucose. Cerebral ischemia is an important event in clinical and surgical neurological practice since it is one of the diseases that most compromise the human species.

Stroke is the third-leading cause of death, and the main cause of disability in the western world. It presents a large socio-economic burden. The etiology can be either ischemic (in the majority of cases) or hemorraghic. The cause of ischemic stroke is often embolic, or thrombotic. So far, there is no effective treatment for the majority of patients suffering from a stroke. Also, there is no effective treatment for subjects who have suffered from stroke that allow neurogenesis of functional neurons. The only clinically proven drugs so far are tissue plasminogen activator (TPA) and Aspirin. Due to the platelet aggregation inhibiting effect of Aspirin only reduced risk of thrombogenesis can be achieved. This effect is not suitable to dissolve an already existing thrombus in the situation of an acute ischemic stroke. Therefore, drugs which solely inhibit the platelet aggregation are merely indicated for the prevention of an ischemic stroke but not for the treatment of an acute ischemic stroke. Furthermore, Aspirin as well as TPA are clearly contraindicated in the case of an hemorrhagic stroke. After massive cell death in the immediate infarct core due to lack of glucose and oxygen, the infarct area subsequently expands, owing to secondary mechanisms such as glutamate excitotoxicity, apoptotic mechanisms, and generation of free radicals. Cerebrovascular disease (CVD) is commonly and frequently encountered, endangering the people's life and work because of its high mortality and serious disability. Cerebral ischemia accounts for seventy percents of CVD.

The mechanism of brain edema formation and secondary neuron injury are not very clear now. Up to now, there is no one kind of mechanisms to explain the damage theorem after cerebral ischemia. Researchers consider that severeal mechanisms participate the process of cerebral infarction: metabolic block, calcium overload, cell apoptosis, the toxic action of excitatory amino acids and free radicle, the nerve damage mediated by nitrogen monoxidum. India is ranked among the countries where the information on stroke is minimal and therefore there is a need to initiate steps to collect data on morbidity and mortality due to stroke in the country as first step towards control measures. The prevalence of stroke is estimated as 203 per 100,000 populations above 20 years amounting to a total estimate of about 1 million cases. The male to female ratio is 1.7. Around 12% of all strokes occur in population below 40 years. The total number of deaths due to stroke is about 102,000,
which represents 1.2% of total deaths in the country .

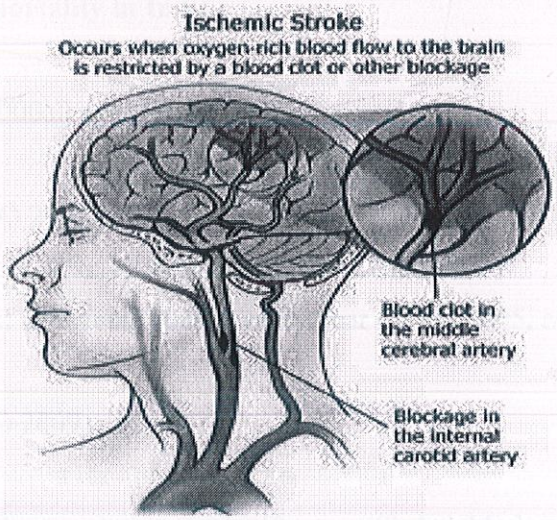

Figure 1: Diagramatic representation of Ischemic Stroke

MORBIDITY AND MORTALITY ASSOCIATED WITH STROKE

Global Stroke morbidty and Mortality

400-800 stroke per 100,000 (Banarjee 2005)

5.7 million deaths (Sridharan 2006)

15 million new acute strokes every year every (Shah + Mathur2006)

28,500,000 DALYs (disability adjusted life-year) (WHO 2004)

28-30 days case fatality ranges from 17%-35% (Feigin et al 2009)

Stroke morbidity and mortality in India

Prevalence 55.6 per 100,000all ages (Dalal 2007)

0.63 million deaths (WHO 2005)

1.44-1.64 million cases of new acute strokes every year (WHO 2005, Murthy 2007)

6,398,000 DALYs (WHO 2009)

12% of strokes occur in the population aged <40 years (Shah + Mathur 2006)

28-30 day case fatality ranges from 18-41% (Dalal et al 2008, Das et al 200

Table1: Death from cerebrovascular disease in India China and established market economies (EME) in millions (Abridged from Ezzati et al 2004

1990			2000			2010			2020		
India	China	EME	India	China	EME	India	China	EME	India	China	EME
0.45	1.27	0.79	0.6	1.65	0.87	0.75	1.91	0.88	0.95	2.29	0.91

Table 2 : Risk factors of stroke among the Indian Population

	Prevalence of risk factors,India	Odda ratio, India			Odds ratio, US	PARPIndia
Study	Tripathi all ages	Bhattachaya all ages	Lipsaka<45 years	Zodpey all ages	AHA all ages	Zodpey all ages
Alcohol consumption	22.5%			1.96	1.8	0.09
Diabetes	3%-12%	1.73		2.39	1.8	
Family history of stroke	8%					
Heart disease	7% with AF	6.20		3.4	1.73	
High cholesterol	7%-32%			2.27	2.0	0.14
Hypertension	12-40%	2.79		1.99	1.0-4.0	0.17
Obesity	6%-49%			1.91	1.75-2.37	
Past historyof TIA				8.44		0.08
Smoking	13% (women) 46% (men)	3.92		1.11	1.9	

PARP= population attributable risk proportion; AF= atrial fibillation, Sridharan et al 2009

Symptoms and Signs of Stroke (Bath et al 2000)

Anterior circulation strokes:

Unilateral weakness, sensory loss or inattention Unilateral weakness

Isolated dysarthria

Dysphasia

Vision : Homonymous hemianopsia, monocular blindness , visual inattention

Posterior circulation strokes:

Isolated homonymous hemianopia

Diplopia and disconjugate eyes

Nausea, vomiting, inco-ordination and unsteadiness

Unilateral or bilateral weakness and/or sensory loss

Non- specific signs:

Dysphagia, incontinence, loss of consciousnes

Complication of Stroke (Bath et al 2000)

Hyperglycaemia

Hypertension

Fever

Infarct extension or re-bleeding

Cerebral edema, herniation, coning

Aspiration

Pneumonia

Urinary tract infection

Cardiac dysrhythmia

Recurrence

Deep vein thrombosis, pulmonary embolism

Cerebral Ischemia

Cerebral ischemia is an ischemic condition where the brain or part of the brain do not receive enough blood flow to maintain normal neurological function.

Ischemia develops within one minutes, forming two zones around the sites of thrombosis or embolism and brain cells at the center of ischemic region is completely arrested .

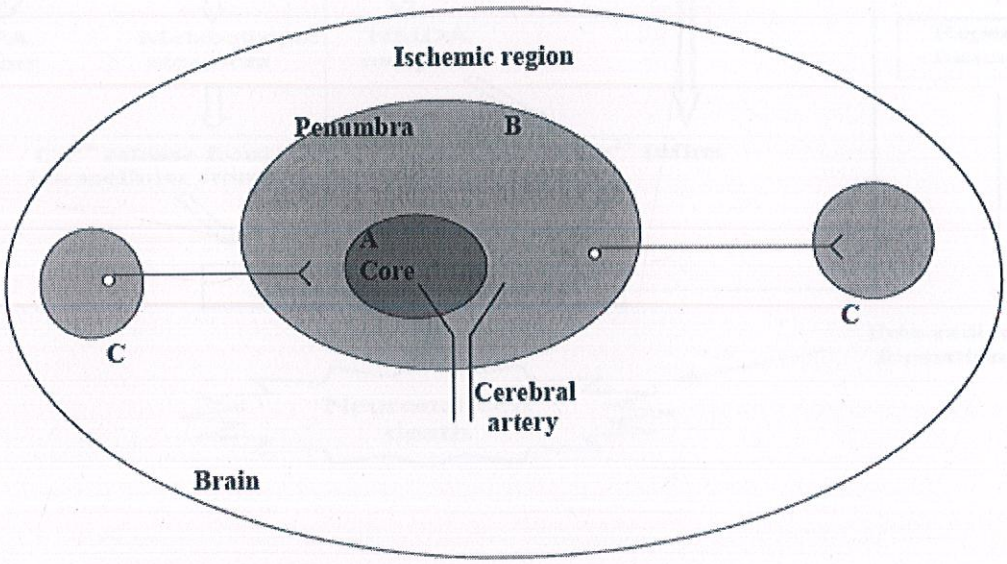

Fig 2: Diagrammatic representation of (A) ischemic core region (B) penumbra area surrounding ischemic core (C) area connected to ischemic region.

Pathophysiology of Cerebral Ischemia

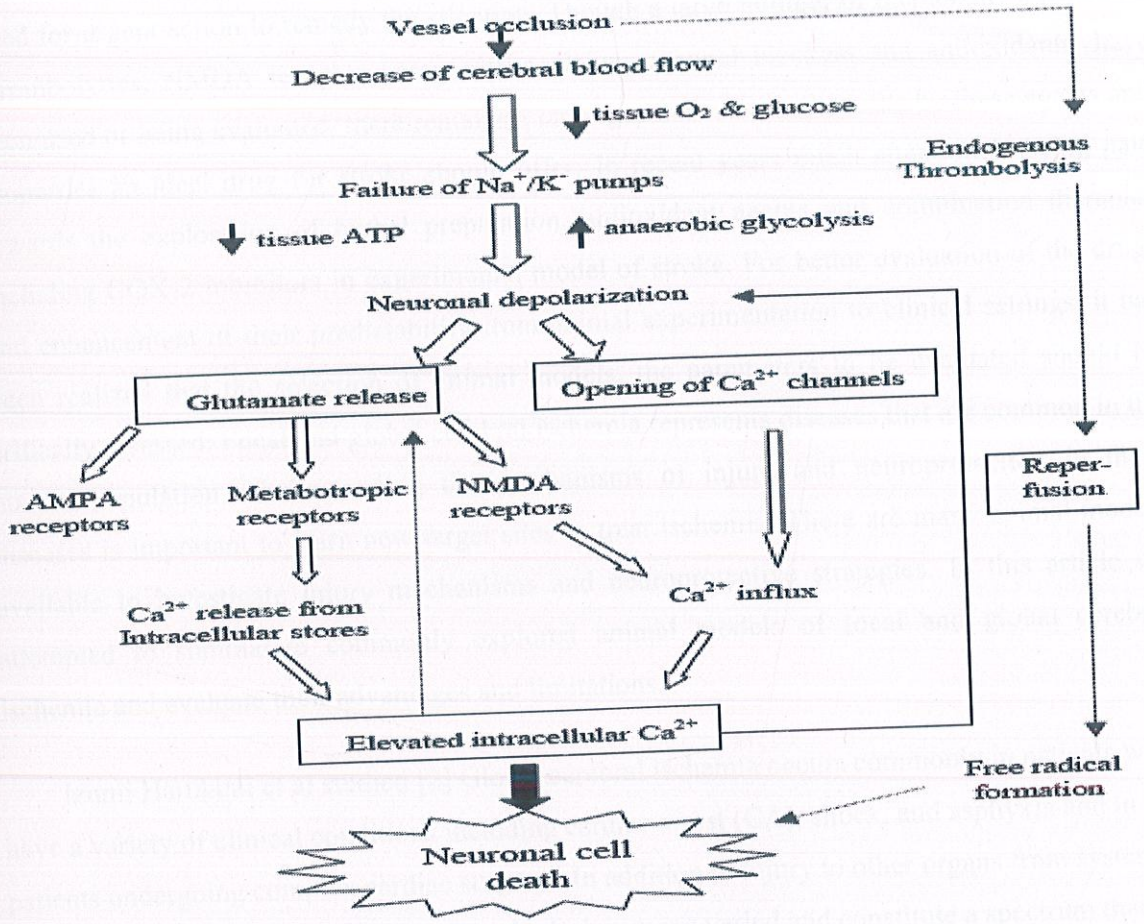

Fig. 3 : Diagrammatic representation of events that may lead to cell death after the onset of cerebral ischemia.

Y.K Gupta studied [1] Stroke is a major cause of death and disability worldwide. The resulting burden on the society continues to grow, with increase in the incidence of stroke. Brain attack is a term introduced to describe the acute presentation of stroke, which emphasizes the need for urgent action to remedy the situation. Though a large number of therapeutic agents like thrombolytics, NMDA receptor antagonists, calcium channel blockers and antioxidants, have been used or being evaluated, there remains a large gap between the benefits by these agents and properties an ideal drug for stroke should offer. In recent years much attention is being paid towards the exploration of herbal preparation, antioxidant agents and combination therapies including COX-2 inhibitors in experimental model of stroke. For better evaluation of the drugs and enhancement of their predictability from animal experimentation to clinical settings, it has been realized that the selection of animal models, the parameters to be evaluated should be critically assessed. Focal and global cerebral ischemia represents diseases that are common in the human population. Understanding the mechanisms of injury and neuroprotection in these diseases is important to learn new target sites to treat ischemia. There are many animal models available to investigate injury mechanisms and neuroprotective strategies. In this article we attempted to summarize commonly explored animal models of focal and global cerebral ischemia and evaluate their advantages and limitations.

Izumi Harukuni et al studied [2] Global cerebral ischemia occurs commonly in patients who have a variety of clinical conditions including cardiac arrest (CA), shock, and asphyxia and in patients undergoing complex cardiac surgery . In addition to injury to other organs from systemic hypoperfusion, neurologic sequelae from brain injury are varied and constitute a spectrum that includes coma, seizures, eschemic stroke, delirium, and neurocognitive impairment.The commonest postulated mechanism for ischemic brain injury after CA (with subsequent resuscitation) is global cerebral ischemia from systemic hypoperfusion that can occur with or without pre-existing large-vessel occlusive disease. Embolism that arises from the heart, from aortic arch artheromas, or from extracorporeal circulation devices occurs more commonly in the perioperative period following complex cardiac surgery and less commonly during resuscitation following CA . Irrespective of the etiology of cerebral ischemia, cellular and molecular processes trigger a cascade of events that culminate in a ''final common pathway,'' resulting in ischemic

neuronal injury. Identification of these injury mediators and pathways in a variety of experimental animal models of global cerebral ischemia has led to investigation of target-specific cytoprotective strategies that are critical to clinical brain injury outcome. Although the authors have previously published on this subject , this article expands on the translational significance of many of the potential neuroprotective strategies that have provided important insights into the mechanism or mechanisms of ischemic brain injury and might be of therapeutic benefit in the future.

Kristian P Doyle et al studied [3] Each year in the United States approximately 700,000 individuals are afflicted with a stroke and currently there are almost 2 million survivors of stroke living in the US with prolonged disability. In China 1.5 million people die from stroke each year and in developed nations stroke is the third leading cause of death, only surpassed by heart disease and cancer. Brain injury following stroke results from the complex interplay of multiple pathways including excitotoxicity, acidotoxicity, ionic imbalance, oxidative/nitrative stress, inflammation, apoptosis and peri-infarct depolarization. Here, we discuss the underlying pathophysiology of this devastating disease and reveal the intertwined pathways that are the target of therapeutic intervention.

Wade S. Smith, MD, Phd Studied [4] pathophysiology of cerebral ischemia is best understood in animal models of stroke. Within minutes of interrupted blood flow, mitochondria are deprived of substrate, which prevents adenosine triphosphate generation and results in membrane depolarization. This leads to increased intracellular calcium and sodium concentration followed by generation of free radicals and initiation of apoptosis. Glutamate release from ischemic neurons contributes to cellular damage. Each step in this complex, interdependent series of events offers a potential point to intervene and prevent neuronal death. Although many human trials in acute stroke therapy have had disappointing results, many promising therapies are in the pipeline, including hypothermia and free-radical inhibitors. Herein, the pathophysiology of focal cerebral ischemia as has been revealed in rodent models and reviews the major human trials according to treatment mechanism.

Richard J. Traystman Studied [5] The use of appropriate animal models is essential to predict the value and effect of therapeutic approaches in human subjects. Focal (stroke) and global (cardiac arrest) cerebral ischemia represents diseases that are common in the human population. Stroke and cardiac arrest, which are major causes of death and disability, affect millions of individuals around the world and are responsible for the leading health care costs of all diseases. Understanding the mechanisms of injury and neuroprotection in these diseases is critical if we are ever to learn new target sites to treat ischemia. There are many animal models available to investigate injury mechanisms and neuroprotective strategies. This review summarizes many (but not all) small and large animal models of focal and global cerebral ischemia and discusses their advantages and disadvantages.

Piperine – The major Alkaloid of *Piper nigrum*

Piperine is a pungent substance found in plants of the Piperaceae family – including *Piper nigrum* (black pepper) and *Piper longum* (long pepper). These peppers have been used in Ayurvedic medicine for the treatment of various diseases and discomforts. Recent research has provided support for some of these uses and has uncovered the probable mechanism responsible for them.

- Increasing the brain's production of beta-endorphins
- Pain relief
- Increasing the brain's production of serotonin
- Anticonvulsant, anti-epileptic action
- Increasing the adrenal glands' production of epinephrine (adrenaline)
- Altering contractions in the upper and lower digestive tract
- Reducing the stomach's production of acid (for about 1 hour)
- Decreasing ulceration of the stomach
- Increasing the pancreas's production of digestive enzymes (amylase, lipase, trypsin and chymotrypsin)
- Stimulating production of melanin
- Reducing inflammation due to irritation or allergy
- Relieving asthma symptoms

B. Bindu madhavi studied [6] Piperine {[1-5-(1, 3)-benzodioxol-5-yl)-1-oxo-2, 4-pentadienyl]-piperidine},an alkaloid responsible for the pungency of black pepper & long pepper. Systemic pharmacological studies on piperine have revealed that this compound elicited diverse pharmacological activities; analgesic, anti-pyretic, anti-inflammatory, anti-convulsant & CNS-depressant activities. Piperine was isolated from *Piper nigrum Linn.* (Piperaceae) and identified by TLC. Piperine was fabricated into alginate beads using sodium alignate. The main aim of this study was to demonstrate the sustained release of piperine from alginate beads by *in vitro* evaluation. The drug release studies were showed that the alginate beads sustained the release of the drug with % drug released in hours.

Srinivasan K. studied [7] Black pepper (Piper nigrum) is one of the most widely used among spices. It is valued for its distinct biting quality attributed to the alkaloid, piperine. Black pepper is used not only in human dietaries but also for a variety of other purposes such as medicinal, as a preservative, and in perfumery. Many physiological effects of black pepper, its extracts, or its major active principle, piperine, have been reported in recent decades. Dietary piperine, by favorably stimulating the digestive enzymes of pancreas, enhances the digestive capacity and significantly reduces the gastrointestinal food transit time. Piperine has been demonstrated in in vitro studies to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Black pepper or piperine treatment has also been evidenced to lower lipid peroxidation in vivo and beneficially influence cellular thiol status, antioxidant molecules and antioxidant enzymes in a number of experimental situations of oxidative stress. The most far-reaching attribute of piperine has been its inhibitory influence on enzymatic drug biotransforming reactions in the liver. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and UDP-glucuronyl transferase. Piperine has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals by this very property. Piperine's bioavailability enhancing property is also partly attributed to increased absorption as a result of its effect on the ultrastructure of intestinal brush border. Although initially there were a few controversial reports regarding its safety as a food additive, such evidence has been questionable, and later studies have established the safety of black pepper or its active principle, piperine, in several animal studies. Piperine, while it is non-genotoxic, has in fact been found to possess anti-mutagenic and anti-tumor influences.

Mittal R , Gupta RL. Studied [8] Oxygen radical injury and lipid peroxidation have been suggested as major causes of atherosclerosis, cancer, liver disease and the aging process. Piperine, having an antiinflammatory effect, has been demonstrated in in vitro experiments to protect against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species and hydroxyl radicals. The effect on lipid peroxidation was also examined and IC50 values were calculated. Piperine was found to act as a hydroxyl radical scavenger at low concentrations, but at higher concentrations, it activated the fenton reaction resulting in increased generation of hydroxyl radicals. Whereas it acts as a powerful superoxide scavenger and IC50 is

1.82 mM, a 52% inhibition of lipid peroxidation was observed at a dose of 1400 microM with an IC50 of 1.23 mM. The results depict that piperine possesses direct antioxidant activity against various free radicals. This study also opens newer views on the potential efficacy of piperine in protecting tissues from peroxidative damage.

Chapter 3

Material and Methods

Methodology:

INDUCTION OF CEREBRAL ISCHEMIA/REPERFUSION INJURY

Materials-

piperine, xylazine, ketamine, povidone iodine solution (betadine), normal saline, atropine

Equipments-

actophotometer, shuttle box

We used swiss albino mice for the induction of cerebral ischemia.

The mice were segregated into 4 groups :

1) NORMAL
2) SHAM OPERATED
3) CEREBRAL ISCHEMIC
4) CEREBRAL ISCHEMIC + PIPERINE

- The cerebral ischemia was induced by occulding both the common carotid arteries with microclips for 20 minutes under ketamine/xylazine anaesthesia.
- The microclips were removed after 20 minutes and reperfusion was allowed for four days.
- At postsurgical case, all the animals were maintained in individual cages and placed in temperature controlled room.
- Betadine was applied topically on surgical wounds.

Behavioral Studies

Actophotometer test-

Animal locomotors behaviour was monitored using actophotometer. Actophotometer is provided with digital counter photocell & a light source was used to measure locomotor activity of animals. Each animal was placed in actophotometer for 10 min & basal activity was recorded for all groups of animals.

Passive Avoidance Step through Task:-

The inhibitory avoidance test, also called passive avoidance, has been used as a screening test to evaluate drug effects on the memory in mice.

The test is based on the natural photophobia of mice or rats, and evaluates the long-term memory of animals The apparatus consists of a box divided into two compartments of equal sizes. One compartment was illuminated with a torch placed on the top of the chamber while the second compartment was kept dark.

The two compartments are separated by a guillotine door.In a trial, the animal was placed in the bright compartment and readily enters the dark compartment. Simultaneously, at that moment, the door separating the two compartments automatically closes, and the animal receives a brief mild electric shock.

During a subsequent trial, the latency to enter the dark compartment was recorded as an index of memory consolidation. The longer the latency to enter the dark compartment, the better the animal is supposed to remember it received an electric shock during a previous trial.

Beam Walking Test:-

Beam walking test is used to assess the coordinated motor activity in mice.

In this test a long thread approximately 1 meter in length was taken and was held from the two sides. The mice will start at the one end, step on to the beam, and walk the entire length of the beam to the other end. The time interval for 1min is noted down; if the mice were able to fall off before the 1 min then it showed the negative results.

Chapter 4

Results

Results

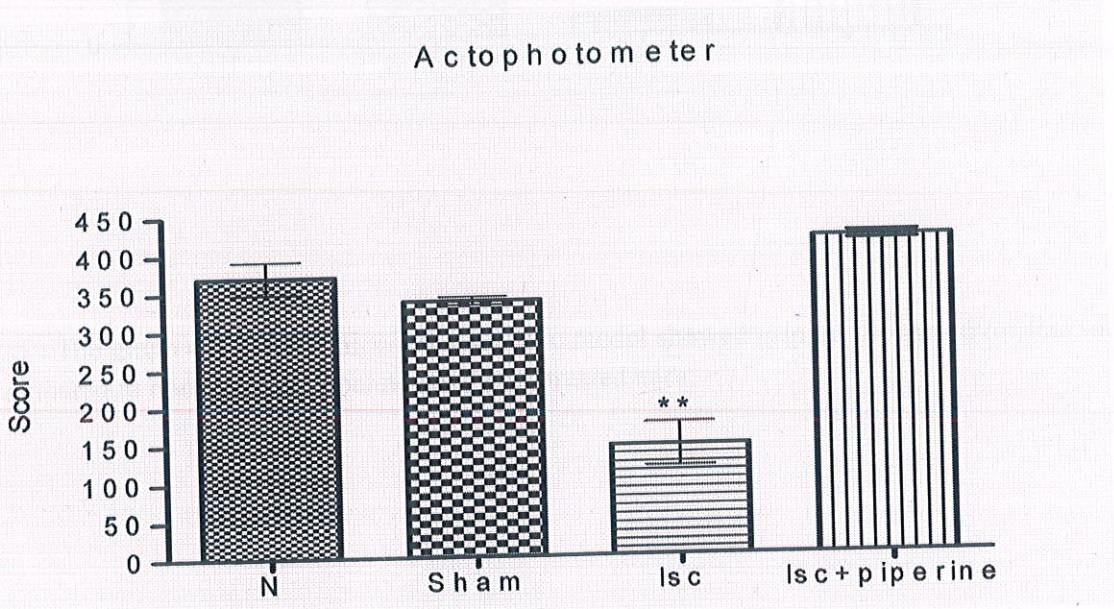

Fig. 4: From the above graph the mice with ischemic condition showed less motor activity as compared to normal, sham and drug treated mice.

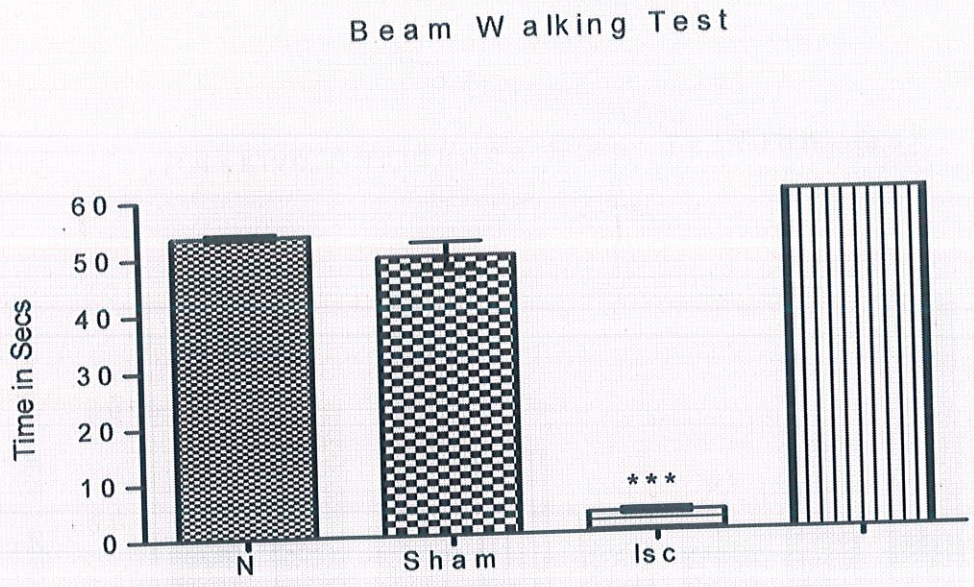

Fig. 5 : The graph shows that mice with ischemic model showed grip on the thread for less time as compared to normal ,sham operated and drug treated mice.

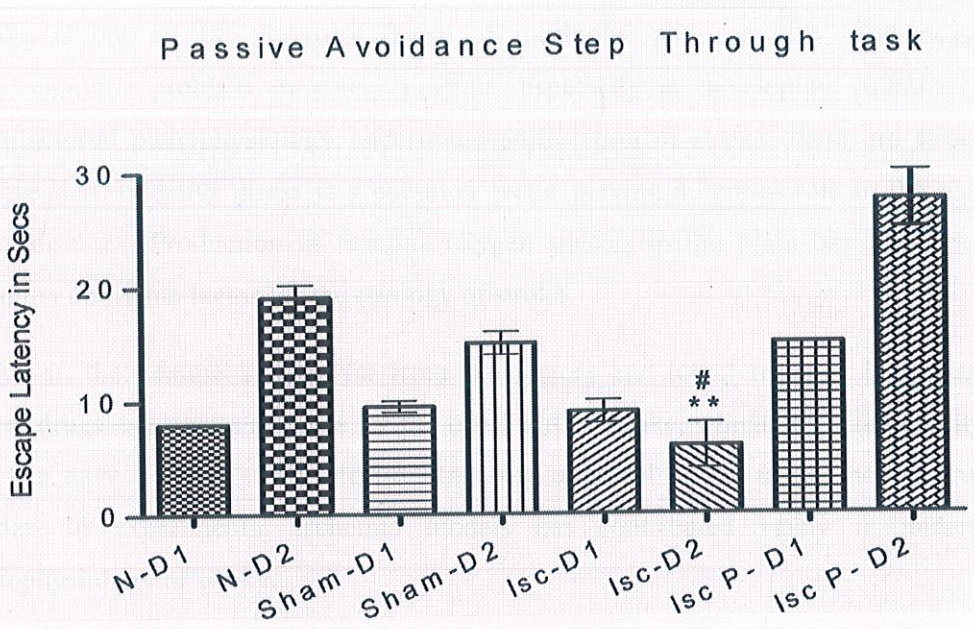

Fig. 6 : From the above graph, the ischemic mice has less latency to enter the compartment as compared to normal , sham operated and drug treated mice .

Discussion

Stroke is one of the foremost cause of morbidity and mortality, and possess a major socioeconomic problem in young patients, especially in developing countries. Despite the multifactorial pathophysiology underline manifestation of stroke, there are strong reasons to believe that oxidative stress is a common factor playing a central role in the pathogenesis of these diseases. Production of reactive oxygen species in the brain has been implicated as a common underline factor for the etiology of stroke.

However, the treatment is yet far from satisfactory and lot of research is undergoing to find newer drugs and newer avenues for the treatment of stroke, which can satisfactorily treat stroke and can have a better safety profile. One such approach is the use of herbal drugs in stroke. Studies in experimental ischemia models has contributed vastly in understanding the pathophysiology of stroke.

Moreover animal models provide a testing ground for novel compound before their launching into any clinical trials. Lot many animal models are available at present , however with the failure of neuroprotective drugs in the clinical trials although being efficacious in experimental studies has led to much concern on the validity of these models and also how closely they mimic human conditions. Therefore it is being suggested that animal models should be refined to best reflect the clinical situation.

Conclusion

Experimental data for different behavioral studies along with the effect of drug in an induced ischemic model of mice have been reported in this dissertation.We studied memory impairment and locomotor activity through various behavioral tests.The piperine attenuated the ischemic induced alteration in behavioural studies in actophotometer, passive avoidance step through and beam walking test.

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