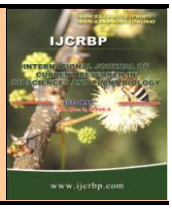
EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 5 (May-2015) pp. 216-225

www.ijcrbp.com



Original Research Article

Pharmacological Studies of *Delonix elata* (L.) Gamble (Caesalpiniaceae)

M.Senthilkumar* and N.Sami Veerappa

Department of Botany, Periyar EVR College (Autonomus), Tiruchriappalli 620 023, Tamil Nadu, India

**Corresponding author.*

Abstract

A number of Indian medicinal plants have been used for thousands of years in the traditional system of medicine (Ayurveda). Amongst these are plants used for the management of neurodegenerative diseases such as Parkinson's, Alzheimer's, loss of memory, degeneration of nerves and other neuronal disorders by the Ayurvedic practitioners. The part of the Ayurvedic system that provides an approach to prevention and treatment of degenerative diseases is known as *Rasayana*, and plants used for this purpose are classed as rejuvenators. In the present study, three such *rasayana* plants were tested for the first time for their toxicity and free radical scavenging activity both *in vitro* and *ex vivo*. Leaf and root of plant extract (up to 1 mg/ml) showed no toxic effects. Neuroblastoma SH-SY5Y cells were maintained in DMEM supplemented with 10% heat- inactivated horse serum and 5% heat inactivated Fatal Bovine Serum (FBS). After pretreatment with *Delonix elata* or Trolox for 30min, H2O2 was added to SH-SY5Y cell cultures for 24 h. To examine how *Delonix elata* acted on serum deprivation-induced cell death, cells were seeded into collagen-coated 24-well plates at a density of 1χ104 cells per well. After incubating for 1 day, cells ere differentiated into neuronal cells as described above. To induce cell death, the differentiated cells were immersed in serum-free DMEM supplemented with 0.1% BSA, and maintained in this condition for 2 days. Leaf and root methanol extracts were tested for their antioxidant activity and free radical cation decolorization assay; inhibition of lipid peroxidation by plant infusions was carried out using spontaneous lipid peroxidation of rat brain homogenate were determined. Result of leaf and root extract of *Delonix elata* play a vital role in the recovery of stroke (brian attack) and its related complications.

Keywords

Antioxidant activity

Delonix elata

Methanolic extracts

Neurotransmitters

SH - SY5Y

Introduction

The birth of neuroscience in India dates back to our independence, when a number of scientists left for training abroad and returned to start nuclei of various neuroscience disciplines in different parts of the country. These pioneers struggled against all odds, gathered bright young people around them and started

service, teaching, training and research. Some of these nuclei developed into full-fledged institutes of neurosciences with all modern facilities. Neuropharmacology appears to have been an area of interest of large number of pharmacologists in the country. Very active groups were established at Lucknow, Varanasi, Bomaby, Baroda, amongst others. The major areas of interest have been basic studies in realation to central mechanisms underlying emetic, analegestic, thermo-regulation and hyponogenic functions, as also drugs acting on higher nervous activity. Neurotransmitters and receptors involved in some of these functions, as also in diseased states, have been evaluated. It is not surprising that there has been an active interest in the area of Indian plants for their medicinal value. More recently there has been some activity in the field of new drug development. To provide a brief glimpse of the activity in the field, only some of these contributions are referred to. Starting with the work of Chopra and his colleagues in early thirties on *Rauwalfia serpentina* and it active alkaloid reserpine, a number of pharmacologists investigated this compound (Chopra et al., 1933, Chatterjee and De, 1960). This could be described as one of the first psychotropic drugs, but more importantly for many years this was a sheet anchor for treatment of hypertension. Some new synthetic hypnotics are described the hypnotic activity of quianazolones (Gujaral et al., 1956).

In recent years scientists at Central Drug Research Institute, Lucknow, have developed a new haloperidol-like neuroleptic (centbutindole) having a novel chemical structure (Nityanand et al., 1976, Dua et al., 1977). Several others compounds are under investigation. Harnath and colleagues did some basic studies on sleep using pharmacological approaches (Harnath and Bhatt, 1971; Harnath and Bhatt, 1977).

Pharmacology can be defined as the study about the effects of chemical substances on the function of living systems. It was born in the mid- 19th century as one of biomedical sciences based on principles of experimentation. Until 19th century, knowledge of the normal and abnormal functioning of body is too rudimentary to provide even and rough basis for understanding drug effects. The motivation of pharmacology came from clinical practice. Rudolf Buchheim created the first pharmacology institute in Estonia in 1847 (Rastogi and Mehrotra, 1969). Early pharmacologists focused their attention on plant derived drugs as morphine, digitaline, quinine, ephedrine and others. The impetus for pharmacology came from the need to improve the outcome of therapeutic intervention by doctors those who were skilled at clinical observation and diagnosis but broadly ineffectual in treatment. Until 19th century, knowledge of the normal and abnormal functioning of body was too rudimentary to provide even and rough basis for understanding drug effects. The motivation for pharmacology came from clinical practice, but the science could only be built on the basis of secure foundations in physiology, pathology and chemistry. Rudolf Buchheim created the first pharmacology institute in Estonia in 1847 (Rang et al., 2003). In its beginnings, pharmacology concerned itself exclusively with understanding the effects of natural substances, mainly of plant extracts. An early development in chemistry was the purification of active compounds from plants. Beginning in the 20th Century, the fresh wind of synthetic chemistry began to revolutionize the pharmaceutical industry and with it in the science and pharmacology. The enormous growth of synthetic chemistry in the first half of the 20th century and the resurgence of natural product chemistry caused a dramatic revitalization of therapeutics. Each new drug class that emerged gave pharmacologists a new challenge, and it was then that pharmacology really established its identity and its status among biomedical sciences. The boundaries of pharmacology, as with other biomedical disciplines, are not shapely defined. Within the main subject fall a number of components as neuropharmacology, immune pharmacology and pharmacokinetics (Caius, 1986). The importance of medicinal plants in the treatment of human ailments has been immense. In today's modern age of advancement in instrumentation technology, sophisticated techniques like NMR and MS, have markedly reduced the time and effort required for structural determination. The development of science of phyto pharmaceutics and the hopes for remedies in chronic diseases generated new enthusiasm in the research workers to develop herbal medicines (Kokate et al., 1999). Nearly 50% of drugs used in medicines are of plant origin.

Materials and methods

Following are the tests and estimations performed in the present study by adopting standard laboratory procedures.

In-vivo pharmacological evaluation

1. Motor activity test
2. Cylinder test
3. Tape removal test
4. Water maze navigation task
5. Place water maze –easy protocol
6. Aversive radial arm maze test

Estimation of neurotransmitters and metabolic enzymes

1. Estimation of acetylcholine enzyme
2. Estimation of glutamine levels
3. Estimation of dopamine levels
4. Estimation of serotonin levels
5. Estimation of monoamine oxidase B levels

Preparation of plant extract

Leaf and root plant materials of *Delonix elata* were dried in shade and pulverized to get a coarse powder. A weighed quantity of the powder (900g) was passed through sieve number 40 and subjected to Methanol solvent extraction at a temperature range of 60 - 80°C respectively. Before and after every extraction the powder bed was completely dried and weighed. The filtrate was evaporated to dryness at 40°C under reduced pressure in a rotary vaccum evaporator. A brownish black waxy residue was obtained. The percentage yield of methanolic extract was 16.53% w/w.

Experimental animals

Colony inbred strains of male albino rats (J.S.S. College of Pharmacy, Udhagai, India) weighing 250-300g, obtained from was used for the pharmacological studies. The animals were kept under standard conditions maintained at 23-25°C, 12 h light/dark cycle and given standard pellet diet (Hindustan lever, Bangalore) provided *ad libitum*. The animals were acclimatized to the laboratory conditions for a week prior to the experimentation and randomly divided into four groups of each ten animals. Principles of animal handling were strictly adhered to and the handling of animals was made under the supervision of animal model approval from obtain was ethics committee, India of the institute. Animal and Cell line culture work carried out in the (J.S.S. College of Pharmacy, Udhagai, India).

Acute toxicity studies

The procedure was followed by using OECD guidelines (organization of economic corporation and development) 423 (acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the substances (Ecobichon, 1997). This procedure results in the use of a specified number of animals while allowing for acceptable data-based scientific conclusion. The method used defined doses (2000 µg/kg body weight) and results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for classification of chemical which cause acute toxicity.

Three male rats 250-300 gm were used for the study, since the herbal extracts are relatively nontoxic, the starting dose level of methanolic leaf (MEDE-L) and root (MEDE-L) extract of *Delonix elata* was selected 2000 µg/kg/bw/p.o. the drug was administered orally to rats which were fasted over night with water *ad libitum* before administration of the drug. Body weights of the rats before and after treatment were noted. Any changes in skin and eyes and mucous membrane and also respiratory, circulatory, autonomic, CNS, somato motor activity, behavior pattern were observed. The onset of toxicity and signs of toxicity if any were also noted.

Study design

Experimental procedure

Experimental protocol for study of cerebral ischemia by Vessel Occlusion method (4VO).

Day 1: Electocauterization of vertebral artery

Male albino rats, weighing 250 to 300 grams each were anesthetized intraperitoneally with a combination of Ketamine (40 to 60 mg/kg) and xylazine (3 to 5 mg/kg) (Fischer, 2009). Forebrain ischemia was induced by four-vessel occlusion. A dorsal neck incision is made from the occipital bone to the second cervical vertebra (about 1 cm in length). The paraspinal muscles are separated to expose the second cervical vertebrae. Under the operating lens the visible vertebral arteries at the second vertebra could be easily

electrocatuerized and completely cut by micro scissors to yield complete cessation of circulation of both vertebral arteries.

Day 2: Occlusion of bilateral common carotid arteries

After 24 h, the animals were subjected to 15 min of forebrain ischemia by making a ventral neck incision of 2 cm length and occluding both common carotid arteries (vagus nerve separated) with vascular clips. Rectal temperature was maintained at close to 37°C with a heating pad during and after ischemia.

Animal groupings

The male albino rats were randomized into 6 different groups (n=6 per group)

Group 1: Animals (positive control) with sham operation (without occlusion) and treated with saline (p.o).

Group 2: Animals with sham operation (without occlusion) and treated with 200 mg/kg of MEDE-Leaves (p.o).

Group 3: Animals with sham operation (without occlusion) and treated with 400 mg/kg of MEDE - Root (p.o).

Group 4: Animals (negative control) with s4 VO and treated with saline (p.o).

Group 5: Animals with 4 VO and treated with 200 mg/kg of MEDE - Leaves (p.o).

Group 6: Animals with 4VO and treated with 400mg/kg of MEDE- Root (p.o.)

Estimation of total proteins

Total protein was estimated in brain using the method described by Lowry et al., (2002) (Table 2).

Histopathology

Seven days after ischemia, rats from each group were anesthetized with sodium Thipentone (100mg/kg). Rats were then transcardially perfused with cold saline followed by 4% formalin in phosphate-buffered saline (0.1M; pH 7.4). The brains were removed from the skull and fixed in the same fixative for 24 h. Thereafter, the brains were embedded in paraffin and 5 µm thick sections were coronally cut at the level of the dorsal hippocampus by a rotator microtome. Tissue sections were stained with hematoxylin and eosin. The slide was photographed under 100x (Fig. 1 a-d; Fig. 2 a-d).

Cell line culture

Neuroblastoma SH- SY5Y Cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated horse serum and 5% heat inactivated Fetal Bovine Serum (FBS). The cell lines were purchased from National Chemical Laboratory (NCL), Pune, India. Cultures were maintained at $37^{o}C$ in a humidified 5% CO_2 atmosphere. To examine the effect of propolis on $0.2mMH_2O_2$- induced cell death, cells were seeded at a density of 2×10^4 cells per well into collagen-coated 24-well plates, prepared by putting hydrochloric acid solution (pH 3.0) containing 30 mg/ml collagen into the wells, and left for 2 h. After incubating the cells for 1 day, they were differentiated into neuronal cells by adding 20ng/ml Nerve Growth Factor (NGF) to the above medium for 3 days. To induce cell death, the differentiated cells were immersed in serum-free DMEM supplemented with 0.1% Bovine Serum Albumin (BSA). After pretreatment with *Delonix elata* or Trolox for 30 min, H_2O_2 was added to SH-SY5Y (Fig. 2 a-d) cell cultures for 24 h. To examine how *Delonix elata* acted on serum deprivation-induced cell death, cells were seeded into collagen-coated 24-well plates at a density of 1χ104 cells per well.

After incubating for 1 day, cells ere differentiated into neuronal cells as described above. To induce cell death, the differentiated cells were immersed in serum-free DMEM supplemented with 0.1% BSA, and maintained in this condition for 2 days.

Cell viability

The evaluate cell survival, we examined the change in fluorescence intensity following cellular reduction of resazurin to resorufin. All experiments were performed in DMEM at 37°C. Cell viability was assessed following immersion in 10% resazurin solution for 3h at 37°C, and fluorescence was recorded at 560/590 nm.

Hoechst 33342 and PI and dual staining

At the end of the cell culture, we added Hoeschst 33342 (ex 350 nm. Em 461 nm) and PI (ex 535 nm, em 617 nm) to the culture medium for 15 min at final concentrations of 8.1 and 1.5mM, respectively. The viable cells were Hoechst 33342-positive and PI negative, whereas dead cells were both Hoechst 33342-positive and PI-positive (Fig. 2 a-d).

Focal cerebral ischemia model in mice

Male adult albino mice, weighing 150 - 200g (Japan SLC), were kept under diurnal lighting conditions. Anesthesia was induced by 2.0% isoflurane and maintained with 1% isoflurane and maintained with 1% isoflurane in 70% N_2O and 30% O_2 using an animal general anesthesia machine (Soft Lander; Sin-ei Industry Co. Ltd., Saitama, Japan), maintaining body temperature between 37.0 and 37.5°C with the aid of heating pad and heating lump. A filament occlusion of the left MCA was performed as described previously. Briefly, the left MCA was occlude using an 8-0 nylon monofilament (Ethicon, Somerville, NJ) coated with a mixture of silicone resin (Xantopren; Bayer Dental, Osaka, Japan). Twenty –four hours after this occlusion, the forebrain was divided into five coronal (2mm) sections using, a mouse brain matrix (RBM-2000C; Activational Systems, Warren, MI), and the sections were stained with 2% TTC. All images of the infarcted areas were saved using a digital camera (Nikon Cool PIX 4500) and quantitated using NIH Image software, calculations being performed as in our previous report.

Brain swelling was calculated according to the following forumula: (infarct volume+ ipsilateral undamaged volume-contralateral volume) × 100/contralateral volume (%). Mice were tested for neurological deficits at 24 h after the occlusion, scoring being as described in our previous report : 0, no observe neurological deficits (normal); 1, failure to extend right forepaw (mild); 2, circling to the contralateral side (moderate); 3, loss of walking or righting reflex (severe). The person doing the scoring was native to the treatment group. *Delonix elata* leaf and root extracted with water, was administered intraperitoneally (i.p.) at dose of 30 or 100 mg/kg (0.1ml/10g) four times (at 2 days, 1 day and 60 min before, and at 4h after the occlusion). *Delonix elata* leaf and root was dissolved in purified water and made fresh daily.

Statistical analysis

Data are presented as the mean±SEM. Statistical comparisons were made by means of a one- or two-way analysis of variance (ANOVA) followed by a Student's *t*- test, Dunnett's test or Mann-Whitney U-test using STAT VIEW, version 5.0 (SAS Institute Inc., Cary, NC). A $p<0.05$ was considered statistically significant.

Results and discussion

The impact of carotid endarterectomy on medical secondary prevention after a stroke or a transient ischemic attack has been reported by Touze et al. (2006). Recommendations for comprehensive stroke centers and a consensus statement from the brain attack coalition are given by Alberts et al. (2005). Clinical contribution of PET neurotransmission imaging in neurological disorders (Garraux and Salmon, 2005). A study by Stephen et al. (2007) reports that the brain acts as a target for inflammatory processes and neuroprotective strategies (Stephen et al., 2007). Preclinical evaluation of pharmacokinetics and safety of melatonin in propylene glycol for intravenous administration has been observed by Cheung et al. (2006).

Anti-Apoptotic Effects of Caspase Inhibitors on Rat Intevertebral Disc Cells (Jong-Beom Park et al., 2006). Pretreatment with melatonin reduces volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model (Pei et al., 2002). The utility of melatonin in reducing cerebral damage resulting from ischemia and reperfusion (Cheung, 2003).Pre-treatment with melatonin reduces volume of cerebral infarction in a permanent middle cerebral artery occlusion stroke model in the rat (Pei et al., 2002). The influence of economic development on the association between education and the risk of acute myocardial infarction and stroke (Chang et al., 2002). Melatonin reduces infarction volume in a photothrombotic stroke model in the wild-type but not cyclooxygenase-1-gene knockout mice (Zou et al., 2006). Melatonin reduces nitric oxide level during ischemia but not blood-brain barrier breakdown during reperfusion in a rat middle cerebral aretery occlusion stroke model (Pei et al., 2003).

Male albino rats, weighing 250 to 300 grams each were anesthetized intraperitoneally with a combination of Ketamine (40 to 60 mg/kg) and xylazine (3 to 5 mg/kg) (Fischer, 2009). Weighed a specific quantity of tissue and was homogenized in HCl Butanol in a cool environment.

The sample was then centrifuged for 10 min at 200 rpm. Supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2ml heptane and 0.02ml HCl. After 10 min, shake the tube and centrifuge under same conditions to separate two phases. Upper organic phase was discarded and the aqueous phase was used for dopamine assay (Schlumpf et al., 1974).

Delonix elata leaf and root extract significantly improved the cerebroprotective effect in cerebral ischemia induced by Four Vessel occlusion in rats and results obtained are as follows:

Effect of MEDE on acetylcholine esterase level

Acetylcholine esterase level was found to be significantly (p<0.01) increased in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) decreased the level of acetylcholine esterase when compared with negative control group (Table 1).

Table 1. Effect of MEDE in the levels of neurotransmitters.

Groups	Acetylcholine esterase (µ M/ min/ g protein)	Monoamine oxidase (μMg^{-1})	Dopamine (pgm mg^{-1} tissue)	Serotonin (pgm mg^{-1} tissue)
Control	17.60 ±0.08	75. 79 ± 0.24	652.0 ±1. 23	8.22. 3 ± 2.2
Ischemia	20.00 ± 0.16 a**	84.40 ± 0.16a**	536 ± 1.90 a**	726.0 ± 2.60 a**
Ischemia + MEDE - L (150mg kg^{-1})	18.50 ± 0.03b**	80.88 ± 0.17b**	590 ± 1.15b**	780.2 ± 1.82b**
Ischemia + MEDE - R (300 mg kg^{-1})	17.60 ± 0.05b**	73.26 ± 0.24b**	642. 2 ± 1.74b**	812.6 ± 2.02b**
Values are expressed as Mean ± SEM of 6 animals; Comparisons were between a. Control vs. ischemia and b. ischemia vs treatment groups; ** Represents the statistical significance of p<0.01 done by ANOVA, followed by Dunnets Multiples Comparison Test				

Effect of MEDE on dopamine level

Dopamine level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of dopamine when compared with the negative control group (Table 1).

Effect of MEDE on serotonin level

Serotinin level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of serotonin when compared with the negative control group (Table 2) (Fig. 1 a - d).

Effect of MEDE on MAO-B level

MAO-B level was found to be significantly (p<0.01) increased in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) decreased the level of MAO-B when compared with negative control group.

Effect of MEDE on superoxide dismutase (SOD) level

SOD level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of SOD when compared with the negative control group (Table 2).

Effect of MEDE on catalase (CAT) level

CAT level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of CAT when compared with the negative control group (Table 2).

Effect of MEDE on glutathione peroxidase (GPx) level

GPx level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of GPx when compared with the negative control group.

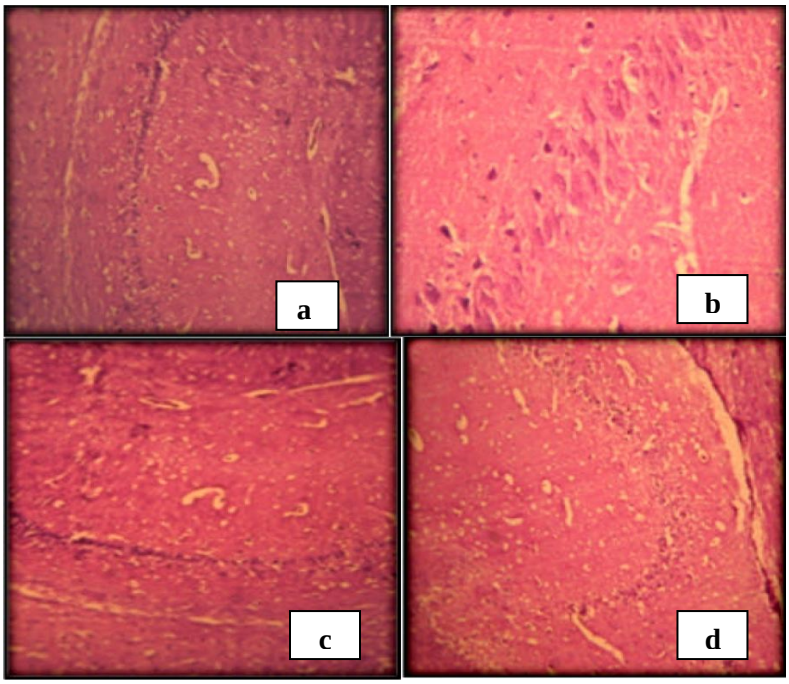
Effect of MEDE on glutathione reductase (GR) level

GR level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of GR when compared with the negative control group.

Table 2. Effect of MEDE in the levels of antioxidants

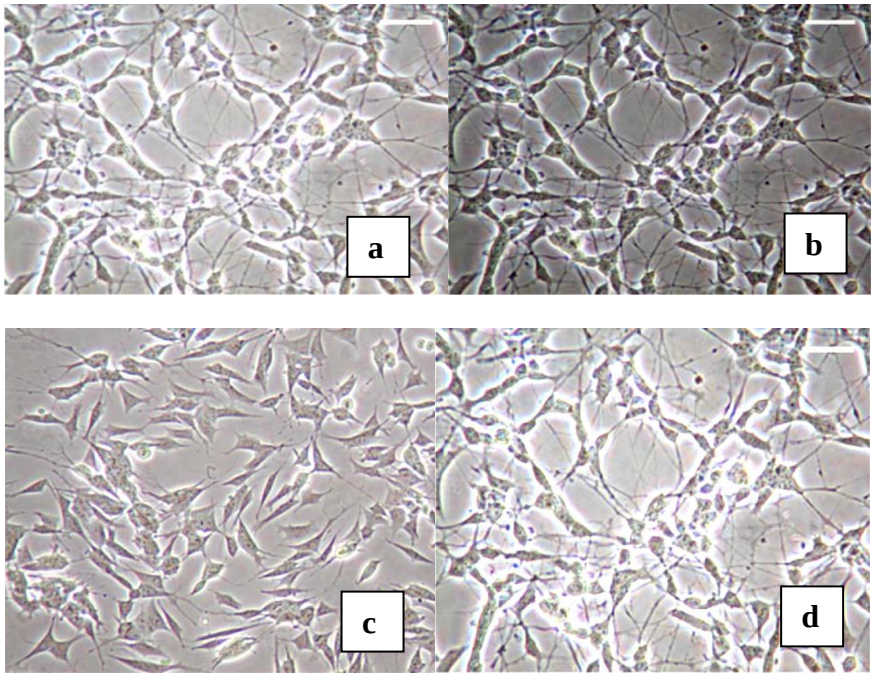
Groups	SOD (units/ mg protein)	Catalase (µmol^{1}/ mg protein/min)	GR (µmol/mg protein/min)	GPx (units/ mg protein)
Control	8.82 ±0.22	36.00 ± 0.24	33.00 ± 0.32	2.40 ± 0.04
Ischemia	6.50 ± 1.16 a**	20.20 ± 0.20a**	22.40 ± 0.18 a**	1.60 ± 0.02 a**
Ischemia + MEDE - L (150mg kg ¯1)	7.00 ± 0.22b**	30.40 ± 0.17 b**	20.10 ± 0.17b**	2.06 ± 0.02b**
Ischemia + MEDE - R (300 mg kg ¯1)	8.30 ± 0.13b**	38.26 ± 0.20b**	32.50 ± 0.26b**	2.48 ± 0.02b**
Values are expressed as Mean ± SEM of 6 animals; Comparisons were between a. Control vs. ischemia and b. ischemia vs treatment groups; ** Represents the statistical significance of p<0.01 done by ANOVA, followed by Dunnets Multiples Comparison Test				

Fig. 1 a - d: Effect of MEDE Leaf and Root on the CA1 hippocampal area 7 days after ischemia.



a. Coronal section showing intact neurons in the hippocampal CA1 region of the control rats.
b. Most pyramidal cells died in the CA1 area 7 days following reperfusion in rats subjected to 10 min ischemia.
c. Administration MEDE-L 150mg/kg/day for 7 days shows the revival of neurons in the pyramidal cells in CA1 subfield.
d. Administration MEDE-R 350mg/kg/day for 7 days shows the revival of neurons in the pyramidal cells in CA1 subfield.

Fig.2 a - d: Effect of MEDE Leaf and Root on the SH-SY5Y Cells cultured in 10% complete medium.



a. Differentiated cells showing normal structure.
b. Differentiated cells treated with 6-OHDA shows the stress signaling.
c. Cells treated with MEDE - L for 3 days showing the revival and survival of cells.
d. Cells treated with MEDE - R for 3 days showing the revival and survival of cells.

Effect of MEDE on protein level

Total protein level was found to be significantly (p<0.01) reduced in the negative control group when compared with the sham operated group. The MEDE treated groups 150mg/kg and 300mg/kg significantly (p<0.01) increased the level of Total protein when compared with the negative control group.

Effect of MEDE on hippocampal cell death

Histopathological studies revealed significant degeneration in hippocampal region of stroke induced group. The treated groups showed significant inhibition of degeneration and signs of regeneration were visible in the groups treated with 150mg/kg and 300mg/kg of MEDE.

Conclusion

Assessing the burden of stroke requires rigorous measurement of stroke incidence, prevalence, mortality and outcome. Although stroke incidence and mortality declined appears to have slowed. An impending epidemic of stroke in the developing world is likely as a urbanization and industrialization that is occurring in many developing regions. Stroke is a disease that has devastating effects on the stroke sufferer, their families and the community. Because stroke is associated with high economic costs the ability for already strained health care systems to cope with the increasing burden is of great concern. Aggressive primary and secondary prevention strategies may reduce some of this burden, and the impact of such public health interventions is potentially greatest in developing nations. Leaf and root extract of *Delonix elata* play a vital role in the recovery of stroke (brain attack) and its related complications.

References

Abbott Alberts, M.J., Richard, E., Latchaw, Warren, R., Selman, Timothy Shephard, R.N., Mark, N., Hadley, Lawrence, M., Brass, Walter Koroshetz, John, R., Marler, John Booss, Richard, D., Zorowitz, Janet, B., Croft, Ellen Magnis, Diane Mulligan, Andrew Jagoda, Robert, O., Connor,

Michael Cawley, Connors, J.J., Jean, A., Rose-DeRenzy, C.N., Martin Emr, R.N., Margo Warren. (2005) Recommendations for comprehensive stroke centers. Stroke. 36, 1597-1618.

Caius, S.J., 1986. The medicinal and poisonous plants of India. Scientific Publishers. Jodhpur, India. pp. 302 -307.

Chang, C.-L., Michael, G., Marmot, Timothy, M.M., Farley, Neil, Poulter, R.2002. The influence of economic development on the association between education and the risk of acute myocardial infarction and stroke. Journal of Clinical Epidemiology. 55, 741-747.

Chatterjee, M.L., De M.S., 1960. Effect of different alkaloids of *Rauwolfia serpentina* on the catecholamine content of adrenal glands of cats. Bull. Calcutta School Trop. Med. 8, 153 -155.

Cheung, R.T.F., 2003. The utility of melatonin in reducing Cerebral damage resulting from ischemia and reperfusion. J. Pineal Res. 34, 153-160.

Cheung, R.T.F., George, L., Tipoe, S. T., Ma, E.S.K., Zou, L.Y., Chan, P.S., 2006. Preclinical evaluation of pharmacokinetics and safety of melatonin in propylene glycol for intravenous administration. J. Pineal Res. 41,337 - 343.

Chopra, R.N., Gupta, J.C., Mukerji, B., 1933. The pharmacological action of an alkaloid obtained from Rauwolfia serpentine Benth. Indian J. Med. Res. 21, 261-271.

Dua, P.R., Raghubir, R., Dhawan, B.N., 1977. Effect of centbutindole on the neuronal activity in the caudate nucleus. Indian J. Pharmacol. 9, 100 - 107.

Ecobichon, D.J., 1997. The Basis of Toxicology Testing, 3rd Edition New York, CRC Press. 43.

Fischer, Y.W., 2008. Manual of Stroke Models in Rats. Published by Taylor Francis (CRC Press). 3, 1- 352.

Garraux, G., Salmon, E., 2005. Clinical contribution of PET neurotransmission imaging in neurological disorders). Acta Neurol. Belg. 105, 1-18.

Gujaral, M.L., Kohli, R.P., Saxena, K.N., 1956. Experimental study of hypnotic potency, toxicity and safety margins of quinazolones (A new series of hypnotics). Indian J. Med. Sci. 10, 871-879.

Harnath, P.S.R.K., Bhat, H.V., 1977. Sleep induced by drugs injected into the inferior horn of the lateral cerebral verntricle in dogs. Br. J. Pharmacol. 59, 231-236.

Harnath, P.S.R.K., Indiranarayan, G., 1971. Sleep induced by cholinomimetic drugs and their antagonists into the vertebral artery in dogs. Indian J. Physiol. Pharmacol. 15, 58-59.

Jong-Beom Park, In-Chul Park, Sung-Jin Park, Hyeon-Ok Jin, Jin-Kyung Lee, Daniel, R. K., 2006. Anti-Apototic Effects of Caspase Inhibitors on Rat Intervertebral Disc Cells. J.Bone Joint Surg. Am.88, 771-779.

Kokate, C.K., Purohit, A.P., Gokhale, S.B., 1999. Pharmacognosy, Nirali Prakashan, Pune. pp 90-93.

Lowry, O.H., Rosehmugh, N.J., Farr, A.L., Randall, K.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265-275.

Nityanand, S., Kapoor, N.K., Dhawan, B.N., 1976. Some observations on the turnover of biogenic amines in the brain with centlentindele a new major tranquilizer (neuroleptic). Indian J. Pharmacol. 8, 71-75.

Pei, Z., Fung, P.C.W., Cheung, R.T.F., 2003. Melatonin reduces nitric oxide level during ischemia but not blood-brain barrier breakdown during reperfusion in a rat middle cerebral aretery occlusion stroke model. J. Pineal Res. 34, 110-118.

Pei, Z., Pang, S.F., Cheung, R.T.F., 2002. Pretreatment with melatonin reduces volume of cerebral infarction in a rat middle cerebral artery occlusion stroke model. J. Pineal Res. 32, 168-172.

Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K., 2003. Pharmacology. 5th Edn., Churchill Livingstone, Edinburgh. pp 352-366.

Rastogi, S., Mehrotra, N., 1969. Compendium of Indian Medicinal Plants, Vol. 1 (1960 – 1969). New Delhi.

Schlumpf, M. L., Langemann, W., Waser, H., Hefti, P.G. F., 1974. A flurometric for the simultaneous determination of serotonin, nordrenaline and dopamine in milligram amounts ofbrain tissue. Biochem Pharmacol. 23, 2337- 2346.

Stephen, D. S., 2007. The brain as a target for inflammatory processes and neuroprotective strategies. Ann. Acad. Sci. 1122, 23-34.

Touze, E., Jean-Louis Mas, Rother, J., Goto, S., Alan, T., Hirsch, Yasuo Ikeda, Chiau-Suong

Liau, Magnus Ohman, M., Alain, J., Richard, P. W.F., Wilson, G. S., P., Bhatt, D.L., 2006. Impact of carotid dndarterectomy on medical secondary prevention after a stroke or a transient ischemic attack. Stroke 37, 2880-2885.

Zou, L.Y., Cheung, R.T.F., Liu, S., Li, G., Li, H., 2006. Melatonin reduces infarction volume in a photothrombotic stroke model in the wild-type but not cyclooxygenase-1-gene knockout mice. J. Pineal Res. 41, 150-156.