International Journal of

Anatomical Sciences

(Official Journal of Association of Anatomists, Tamil Nadu, India)
Volume 5 No. 2 September 2014

www.ijas.in

Editorial Board

Advisory Board

Published By

Association of Anatomists, Tamil Nadu, India
Regd. Under Societies Act, Government of Tamil Nadu – Reg. No:144/1988
Editorial Office, Department of Anatomy, University of Madras
Taramani Campus, Chennai 600 113, India
Phone: 91-44-24547020 Email: editor.ijas@gmail.com

International Journal of Anatomical Sciences
(Official Journal of Association of Anatomists, Tamil Nadu)
September 2014; Volume 5 Issue 2

Contents **Page**

International Journal of Anatomical Sciences 2014, 5(2): 43-45.

Research Article

Morphometric Study of the Foramen Magnum in Adult Human Skulls in South Indian Population

Sumana R, Kirubhanand C, Shradha Iddalgave,

Department of Anatomy, Karuna Medical College, Vilayodi, Palakkad- 678 103, Kerala, India.

Key Words: foramen magnum, morphology, morphometry, skull

Abstract: The morphometric evaluation of foramen magnum is interesting due to its relation with its contents. The aim of the present study was to conduct a morphometric analysis of foramen magnum. Completely ossified 100 adult human dry skulls of unknown age and sex were taken primarily from the Department of Anatomy of Karuna Medical College, Vilayodi, Palakkad were used for the study.

Foramen magnum is a Latin word meaning largest aperture in the skull. Foramen magnum is the most conspicuous feature of the cranial base. The four parts of occipital bones are forming its boundaries. The major structures passing through this large foramen are medulla oblongata with the meninges, vertebral arteries, anterior and posterior spinal arteries and accessory nerves. Foramen magnum can be used for sex determination of the individuals. Hence in the present study, morphometric and morphological analysis of foramen magnum and its variations in the shape in human skull bones was taken.Many authors have classified foramen magnum depending upon its shapes, such as oval, egg shaped, and round, tetragonal, pentagonal, hexagonal and irregular

Materials and Methods

The skull samples which were deformed were excluded from the study. All the 100 cranial bases were visually assessed for foramen magnum shape classification. Each foramen magnum was classified into one of the four shapes, oval, round, irregular, tetragonal, pentagonal and hexagonal. The antero-posterior and transverse diameters were measured using a vernier caliper graded upto 0.01mm. Observations made were tabulated and photographed.

Observations

In the present study oval shaped foramen magnum were found more frequently in female. In the study done, the Round shaped foramen magnums were more frequent in both sexes (Table 1 & Fig. 1).

Table 1 Incidences of various shapes of foramen magnum

SHAPE OF THE FORAMEN	MALE PERCENTAGE IN 100	FEMALE PERCENTAGE IN 100
Oval	10.7%	14.29%
Egg	17.86%	14.29%
Round	39.29%	28.57%
Tetragonal	7.14%	0.00%
Pentagonal	3.57%	28.57%
Hexagonal	10.71%	14.29%
Irregular	0.00%	0.00%

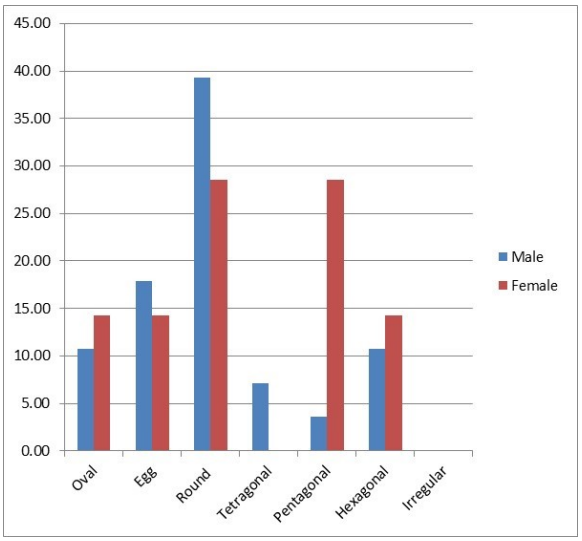
Correspondence to: Kirubhanand C, Department of Anatomy, Karuna Medical College, Vilayodi, Palakkad-678 103, Kerala, India.

Email: kirubhanand.c@gmail.com

Accepted: 30-Sep-2014

IJAS 2015, 5(2): 43-45

Fig 1 Histogram showing incidences of various shapes of foramen magnum in both the sexes.

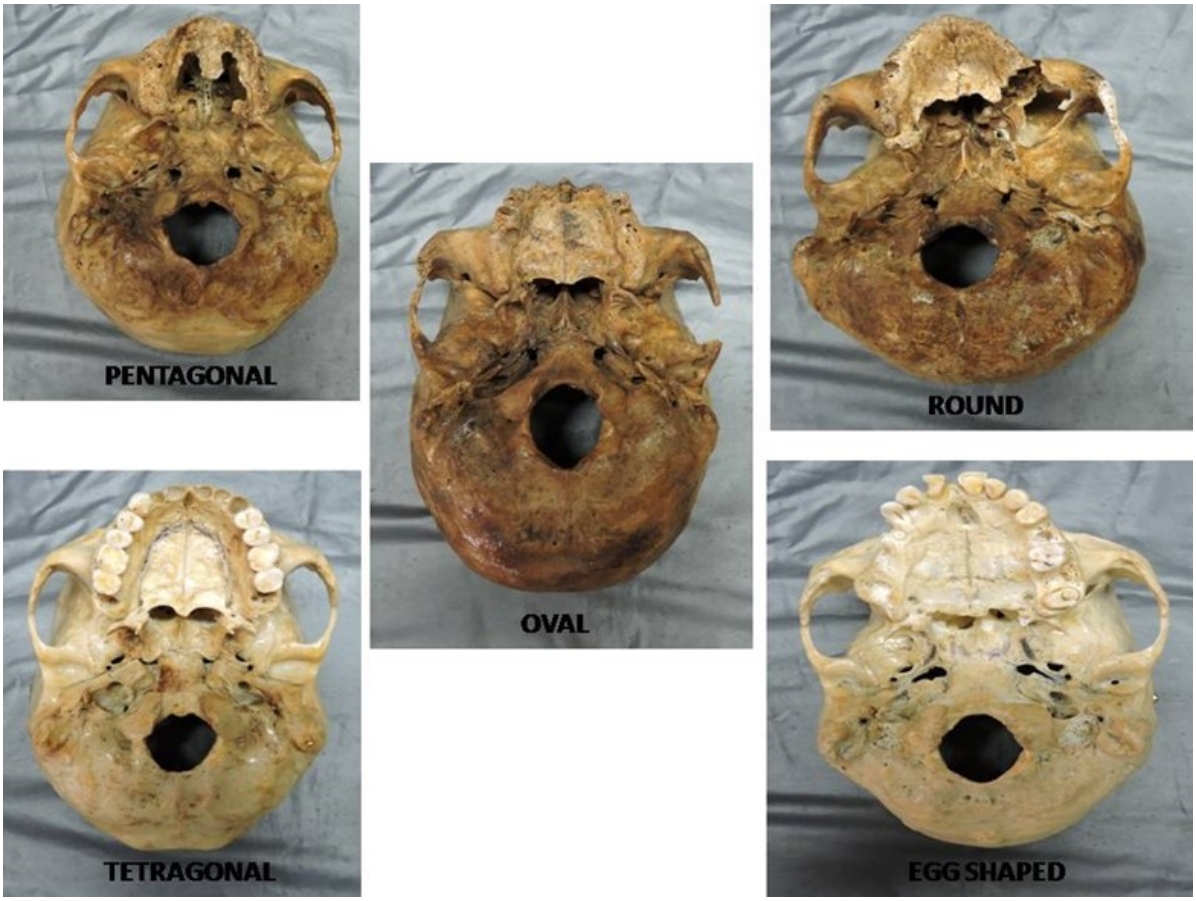


Tetragonal shaped foramen magnum were found only in male. Whereas pentagonal shaped foramen magnum were predominant in females then male. Hexagonal shaped foramen magnum almost equaled the number in both sexes. We did not come across any irregular foramen magnum (Fig 2).

Discussion

The foramen magnum reaches its adult size rather early in childhood and is therefore unlikely to respond to significant secondary sexual changes (Santhosh CS, Vishwanathan KG, Ashok Gupta, Siddesh RC, 2013). From a mechanical point of view, no muscles act upon the shape and size of the foramen magnum and its prime function is to accommodate the passage of structures into and out of the cranial base region and in particular, medulla oblongata which occupies the greatest portion of the foramen space.

Fig 2 Photograph showing various shapes of foramen magnum observed in the study



Population differences are also important in defining sexual differences in the cranium. Therefore sexual differences in the foramen magnum Therefore, it is necessary to know the source population of any unidentified skull and adopt a method based data from that population or a population with similar expression of sexual dimorphism. The LFM and WFM in the South Indian population is a useful indicator of sex, and comparison to values from other populations demonstrates similar results among some of the populations.

The shape and morphological variations of foramen magnum are important in neurological interpretation (Radhika *et al*., 2014). In an ovoid type of the foramen magnum, the surgeon may find it difficult to explore the anterior portion of the foramen magnum. The morphometric analysis of foramen magnum and its variations is important not only anatomists but also to the anesthetist, neurosurgeons, orthopedicians, radiologists.

The sex determination of incomplete or damaged skeletons is an important task in forensic medicine (Santhosh *et al.*, 2013). Anthropometric measurements can aid in solving such problems of gender identification. The foramen magnum was used since it is a regular structure and less likely to major morphological changes.

Conclusion

Hence, it can be concluded that careful radiological analysis of foramen magnum is required before craniovertebral junction surgery to prevent inadvertent complications such as hemorrhage, atlanto occipital instability and injury to major structures passing through foramen magnum. The sexual dimorphism of foramen magnum dimensions is established in the study. However, due to considerable overlapping of male and female values, it is unwise to singularly rely on the foramen measurements. However, considering the high sex predictability percentage of their dimensions in the present study and the studies preceding it, the foramen measurements can be used to supplement other sexing evidence available so as to precisely ascertain the sex of the skeleton.

References

Radhika PM, Shailaja S, Prathap KJ, Sheshgiri C (2014) Morphometric Study of The Foramen Magnum In Adult Human Skulls In Indian Population. Asian J Med Clin Sci 3:68–72.

Santhosh CS, Vishwanathan KG, Ashok Gupta, Siddesh RC and TJ (2013) Morphometry of the Foramen Magnum: An Important Tool in Sex Determination. Res Rev J Med Heal Sci 2:88–91

International Journal of Anatomical Sciences 2014, 5(2): 46-48.

Case Report

Lumbar hernia- A cadaveric Case Study

Kirubhanand C, Tamil Selvi P,[*] Vijaya Prakash KM,[‡] Shradha Iddalgave,

Department of Anatomy, Karuna Medical College, Vilayodi, Palakkad- 678 103, Kerala, India.
[*]Department of Anatomy, Sathyabama Dental College & Hospital, Sathyabama University, Chennai 600 119, Tamil Nadu, India.
[‡]Department of Anatomy, Dr. ALM PGIBMS, University of Madras, Taramani Campus, Chennai 600 113

Key Words: lumbar hernia, petits triangle

Abstract: In this article we report a case of cadaveric finding of inferior lumbar hernia. Lumbar triangle hernia that occurs through lumbar triangles is very rare type of hernia. Only about 300 cases have been reported till date. Petit's triangle hernia find further rarity and the case under reference is probably the first ever reported case of Petit's triangle hernia in cadaveric finding. The relevant literature has been reviewed and the case report is discussed in brief.

Lumbar hernias are quite uncommon as compared to other ventral abdominal wall hernias, accounting for less than 1.5% of all abdominal hernias, with fewer than 300 cases reported over the past 300 years. About 25% of all lumbar hernias have a traumatic etiology (Bhasin and Khan, 2006). Lumbar hernia can occur individually or in association with certain syndromes or following trauma. This may be post-surgical or following blunt injuries associated with intra-abdominal injuries (Walgamage *et al.*, 2015). Clinical diagnosis of this entity is difficult due to non-specific symptoms. The diagnosis is particularly elusive in obese individuals or in post-surgical patients. Though rare defects, lumbar hernias are prone to incarceration and strangulation (Grauls *et al.*, 2004).

The swelling may appear on coughing and disappear on compressing it. On clinical examination there may be single circular/oval swelling of 10 x 8 cms arising from the inferior lumbar triangle with an expansile impulse on pressurizing over lateral abdominal wall. It may be tender or non-tender and reducible on compression (Michael and Richardson, 2012).

Lumbar hernias are rare defects that involve the extrusion of retroperitoneal fat or viscera through a weakness in the posterolateral abdominal wall. Within this region there are two anatomically defined weaker triangles, the triangle of Petit and the triangle of Grynfelt-Lesshaf. The "triangle of Petit" or "the inferior lumbar triangle" is an upright triangle bound by the crista ilica, the musculus obliquus externus and the musculus latissimus dorsi (Singh and Kumar, 2014). The superior lumbar triangle is an inverted triangle bordered by the 12th rib, the musculus serratus posterior inferior, the musculus quadratus lumborum, the musculus erector spinae and the musculus obliquus internus. Lumbar hernia may be asymptomatic, associated with a sense of discomfort, or the cause of notable localized tenderness. - lumbar hernias are rare, the differential diagnosis must be made with a lipoma, a soft tissue tumor, a hematoma, an abscess, an

Correspondence to: Kirubhanand C, Department of Anatomy, Karuna Medical College, Vilayodi, Palakkad-678 103, Kerala, India.

Email: kirubhanand.c@gmail.com

Accepted: 30-Sep-2014

atheromatous cyst, a renal tumor, a panniculitis and a muscle hernia(Mingolla and Amelio, 2009). Repairing these lumbar hernias is often difficult because of the weakness of the surrounding structures

The etiology of a lumbar hernia may be congenital (mal development or malformation of musculo skeltal system) or acquired. The spontaneous acquired variety may represent either a delayed presentation of the congenital variety or may be due to weakening of the muscle layer and various straining factors (Munhoz *et al.*, 2015). In addition 25% of all lumbar hernias have traumatic etiology. This may be post-surgical especially after kidney operation, harvesting a bone graft from the iliac crest, or fashioning a latissimus dorsi flaps. Lumbar hernias may also follow blunt or penetrating injuries to the flanks in which case hernia may be large and not conform to the anatomical boundaries of the lumbar region. Most of the primary lumbar triangle hernias occur through the inferior lumbar triangle of Petit's.

Case Report

Under routine cadaver dissection for undergraduate students in *department of anatomy Karuna medical college Vilayodi, Palakkad 678103, Kerala, India.* The present case is of a 76 years old, male who presented subsequent notice of swelling on side of low back was found. Initially it was thought to be subcutaneous lipoma and on dissection and exploration it turned out to be rarest extra peritoneal Petit's triangle hernia.

Discussion

Lumbar hernias occur more commonly in males and are twice as common on the left as the right side. Patients are usually between 50 to 70 years old. These hernias can occur anywhere within the lumbar region but are more common through the superior lumbar triangle (of Grynfeltt-Lesshaft. These hernias have a natural history of a gradual increase in size over time and may assume large proportions (Sharma, 2009).

Presence of Lumbar Hernia

The hernia may contain retroperitoneal fat, kidney, colon or less commonly small bowel, omentum, ovary, spleen or appendix. On auscultation, bowel sounds may be audible over the swelling if the hernia contains bowel loops. In obese patients detection of a mass is particularly difficult. Bowel incarceration occurs in 25% but strangulation is rare because of wide hernia neck. Lateral or oblique radiograph of the lumbar region may show gas filled loops of the bowel lying outside the abdominal cavity (Sharma *et al*., 2013). Upper or lower gastrointestinal contrast studies are useful in delineating the herniated bowel segment. In addition, an intravenous urogram may be performed to visualize any displacement of the kidney or ureter into the hernia. Ultrasonography may fail to demonstrate the hernia due to low index of suspicion and presence of fat. CT scan can accurately distinguish the muscular and fascial layers, detect the presence of a defect in these layers, visualize herniated viscera and differentiate a hernia from a hematoma, abscess or soft-tissue tumor. The goal of hernia repair is to eliminate the defect and to construct an elastic and firm abdominal wall that will withstand the stress of daily physical activities. A lumbar hernia should be repaired surgically, as it is prone to both obstruction and strangulation.

A wide variety of techniques have been described for repair of lumbar hernias. These include anatomical closure,

overlapping of the aponeuroses, use of musculo fascial flaps, prosthetic meshes and laparoscopic mesh repair in case of uncomplicated lumbar hernias. Currently, extra-peritoneal mesh repair is considered the optimal treatment for isolated unilateral lumbar hernia. Furthermore, lumbar hernias differ from each other by the contents of its hernia sac. Because lumbar hernias seldom cause strangulation, the prognosis is often good. However, their volume increases progressively and they become more symptomatic(Lillie and Deppert, 2010). The larger the hernia, the more difficult the operation. That is why most of the hernias should be operated as soon as the diagnosis has been made. After the hernia sac and its contents are identified and reduced, the reconstruction of the defect can be performed. This reconstruction is difficult because of the weakness of the surrounding tissues and because of the complicated anatomical boundaries. A preoperative CT- scan should be made, with attention to the colon and the urinary tract.

Conclusion

Symptomatology frequently consists of only lower back pain. Small hernias may be asymptomatic except for a palpable mass. In less than 10% of cases, the onset is acute with bowel obstruction. Anamnesis is helpful for diagnosis in post-traumatic or postsurgical lumbar hernias while in spontaneous adult hernias, misdiagnosis may occur. Clinical suspicion is fundamental to guide imaging diagnosis because extra-peritoneal fat herniated through a wall defect may mimic a lipoma. Computed tomography (CT) or magnetic resonance imaging (MRI) in patients with a suspected hernia can confirm the diagnosis adding information on parietal defect size, hernia content and muscular tropism. In our case since defect was large, so there was no need of CT or MRI. Adequate surgical treatment depends largely on the type and size of the hernia. A single surgeon cannot gain great experience in this pathology but knowledge gained in treatment of other abdominal wall hernias helps in proper planning of surgery. Both open and laparoscopic techniques can be used with good results. Anterior repair is appropriate for repairing recurrent or large defects with a double mesh or a gluteus aponeurosis flap. Laparoscopic repair has been used successfully in different reports with less pain, shortened hospital stay and good cosmetic and functional results. Although a rare pathology, knowledge of lumbar hernia is important to avoid misdiagnosis. In particular, a lumbar or flank mass should always raise suspicion of a lumbar hernia. Ultrasound and CT may confirm the diagnosis. Appropriate surgical treatment should be planned on the basis of etiology and hernia size.

References

Singh M, Kumar A, Nag S (2014) Inferior Lumbar Hernia: Case report. J Dent Med Sci 13:16–18.

Grauls A, Lallemand B, Krick M (2004) The retroperitoneoscopic repair of a lumbar hernia of Petit. Case report and review of literature. Acta Chir Belg 104:330–334.

Lillie GR, Deppert E (2010) Inferior lumbar triangle hernia as a rarely reported cause of low back pain: a report of 4 cases. J Chiropr Med 9:73–76.

Sharma P (2009) Lumbar Hernia. MJAFI 65:178–179.

Michael V, Richardson WS (2012) Lumbar Incisional Hernia Repair After Iliac Crest Bone Graft. Ochsner J 12:80–81.

Pietro MG, Amelio G (2009) Lumbar hernia misdiagnosed as a subcutaneous lipoma: a case report. J Med Case Rep 3:9322.

Munhoz AM, Montag E, Arruda EG, Sturtz G, Gemperli R (2015) Management of giant inferior triangle lumbar hernia (Petit's triangle hernia): A rare complication following delayed breast reconstruction with extended latissimus dorsi myocutaneous flap. Int J Surg Case Rep 5:319–323.

Bhasin SK, Khan AB (2006) Bilateral Petit's Triangle Hernia. JK Sci 8:163–164.

Sharma VM, Akruwala SD, Desai S (2013) A case of inferior lumbar hernia. Int J Res Med Sci 1:33–35.

Walgamage TB, Ramesh BS, Alsawafi Y (2015) Case report and review of lumbar hernia. Int J Surg Case Rep 6C:230–232.

International Journal of Anatomical Sciences 2014, 5(2): 49-56.

Research Article

Nicotine Exposure During Gestation on Neonatal Rat Testis Indicate Possibility of Fertility Impairment at Adulthood

Anitha V, Venkata Lakshmi N,[*] Sumathy G,[‡] Karthik Ganesh M, Prakash S.

Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu, India
[*]Department of Anatomy, Sathyabama University Dental College and Hospital, Chennai, Tamil Nadu, India
[‡]Department of Anatomy, Sree Balaji Dental College and Hospital, Chennai, Tamil Nadu, India

Key Words: nicotine, gestation, teratogen, testis, infertility

Abstract: Nicotine is largely consumed as a component of cigarette during smoking. Nicotine exposure during pregnancy leads to many pregnancy related issues, developmental anomalies and increased possibility of adverse health conditions in the adulthood. Though nicotine use during pregnancy has been studied in human and same were co-related with number of adverse obstetrical and developmental outcomes. However, contributions from animal studies seem to be tremendous and also able to bring about the fact that nicotine alone may be a key chemical responsible for many adverse effects associated with maternal cigarette smoking on the progeny. This study was done using pregnant female Wistar Albino rats to investigate the effect of nicotine administration during individual trimesters and full gestation on testis of the neonates. The male pups were picked from the each litter and were used for this study. Observations showed gross change in testis with a decline in testicular size in full gestation treated group (Nic-Full-Trim) when compare to control, 1st (Nic-I-Trim) and 2nd (Nic-II-Trim) trimester treated rats. Histology of testis reveled seminiferous tubules with disorganized and degenerating cells in the lumen in nicotine administrate during 3rd trimester (Nic-III-Trim). In Nic-Full-Trim group the diameter of the seminiferous tubules and germ cell number were much reduced. The severity of the damage was more obvious in Nic-Full-Trim group which showed much reduced testicular size and tubules, degenerating cells and increased interstitial space with marked collagen deposition when compare to the rest of the nicotine treated groups. Increased Caspase-3 and NFκB expression in nicotine treated groups indicate activation of apoptotic pathway in germ cells. Observations indicate that Nic-III-Trim and Nic-Full-Trim group's male progeny testis were severally affected by nicotine exposure and probably would show impaired fertility at adulthood.

Cigarette smoking which increases the risk of fatal diseases like cardiovascular diseases, coronary artery disease, myocardial infarctions, lung cancers, cerebrovascular incidents, etc. Nicotine is an addictive component of cigarettes and exists at high concentrations in the bloodstream of smokers (Benowitz, 1988). Stoppage of smoking during pregnancy can reduce damage to the developing fetus (Lindley *et al.*, 2000; Pickett *et al.*, 2003). Cigarette smoke is a complex mixture of toxic chemicals including

Correspondence to: Prakash S, Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu, India

Email: seppanprakash@yahoo.com

Accepted: 30-Sep-2014

nicotine, carbon monoxide, and several recognized carcinogens and mutagens. These toxicants are absorbed through the pulmonary vasculature and transported via the blood stream causing cytotoxicity, genotoxicity, and tumorigenicity throughout the body. Nicotine is metabolized primarily by the liver, and to a lesser extent, the lung and kidney, with the primary metabolite being cotinine (Stillman,.*et al.*, 1986; Roger and Abbott, 2003).

Effects of Nicotine on Fetus

Pregnant women are exposed to various teratogens with or without their knowledge. With the modernization, most women are indulge in the habit of smoking and few are exposed to passive smoking at work place, public places etc. The follow-up investigations on these progeny indicate that they would develop infertility, diabetes, obesity, hypertension, neurobehavioral defects, and respiratory dysfunction. Cigarette smoking during pregnancy can lead to numerous obstetrical issues including, fetal growth restriction (Hammoud *et al.*, 2005), placental abruption (Ananth *et al.*, 1999), spontaneous abortion (George *et al.*, 2006), sudden infant death syndrome (SIDS) (Mitchell and Milerad, 2006), placenta previa (Hung *et al.*, 2007), preterm birth (Fantuzzi *et al.*, 2007), stillbirth (Hogberg and Cnattingius, 2007) and low birth weight (Jaddoe *et al.*, 2008).

From the experimental studies it is clearly evident that the active or passive exposure of pregnant women to nicotine toxicity causes adverse teratogenic effects on the various stages of embryonic development, leading to congenital anomalies. Nicotine (and its major metabolite cotinine) crosses the placental barrier and is found in fetal tissues in concentrations that are equal to, or greater than plasma nicotine levels in the mother (Dempsey and Benowitz, 2001; Ilett *et al.*, 2003). In adult male cigarette smoking has also been associated with decreased sperm count, alteration in motility of the sperms, and overall increase in the number of abnormal sperms in humans (Kalikauskas *et al.*, 1985) and decreases the level of testosterone (Sarasin *et al.*, 2003).

The aim of the study was to analyze the deleterious effect of nicotine on testis of rats when exposed during embryonic period. This study was done using pregnant female Wistar Albino rats. Effect of nicotine administration during individual trimesters and full gestation on testis of the neonates, thereby delineate the vulnerable period for developing testis and to make out the germ cell loss in the early development periods. The male pups were picked from the each litter and were used for this study.

Materials and Methods

Animals Used

Female Wistar albino rats (*Rattus norvegicus)* used for this study. This species has very good adaptability, able to survive and breed under wide range of climatic conditions in variety of habitats. Albino rats have been extensively used as an experimental model for a variety of studies all over the world including embryological or developmental studies. With biological research point of view the rat and human share many similarities (Weihe, 1983).

Animal Maintenance

Details of the animal maintenance were given elsewhere (Suresh *et al.*, 2009). The study was approved by the institutional ethical committee (IAEC no.01/004a/06). Animals were maintained according to the guidelines of the Canadian Council for Experimental Animal Care (1993) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (2003) guidelines for laboratory animal facility. Healthy female animals were randomly sorted into different five groups (*vide infra*). These rats were allowed to mate with a healthy proven fertile male rat. After confirming the pregnancy, these rats were subjected to nicotine hydrogen tartrate (Sigma-Aldrich, USA) administration at a daily dosage of 2 mg/kg of

body weight (i.p.). Details of the animal grouping as follows,

1. Group I - Control
   Nicotine Administrated groups
2. Group II - During 1st week of gestation i.e. 1 -7 days (Nic-I-Trim)
3. Group III - During 2nd week of gestation i.e. 8 - 14 days (Nic-II-Trim)
4. Group IV - During 3rd week of gestation i.e. 15 - 21 days (Nic-III-Trim)
5. Group V - Full period of gestation i.e. for 21 days (Nic-Full-Trim)

The male pups from corresponding groups were selected from each litter. These pups were used for this study.

Histological Analysis

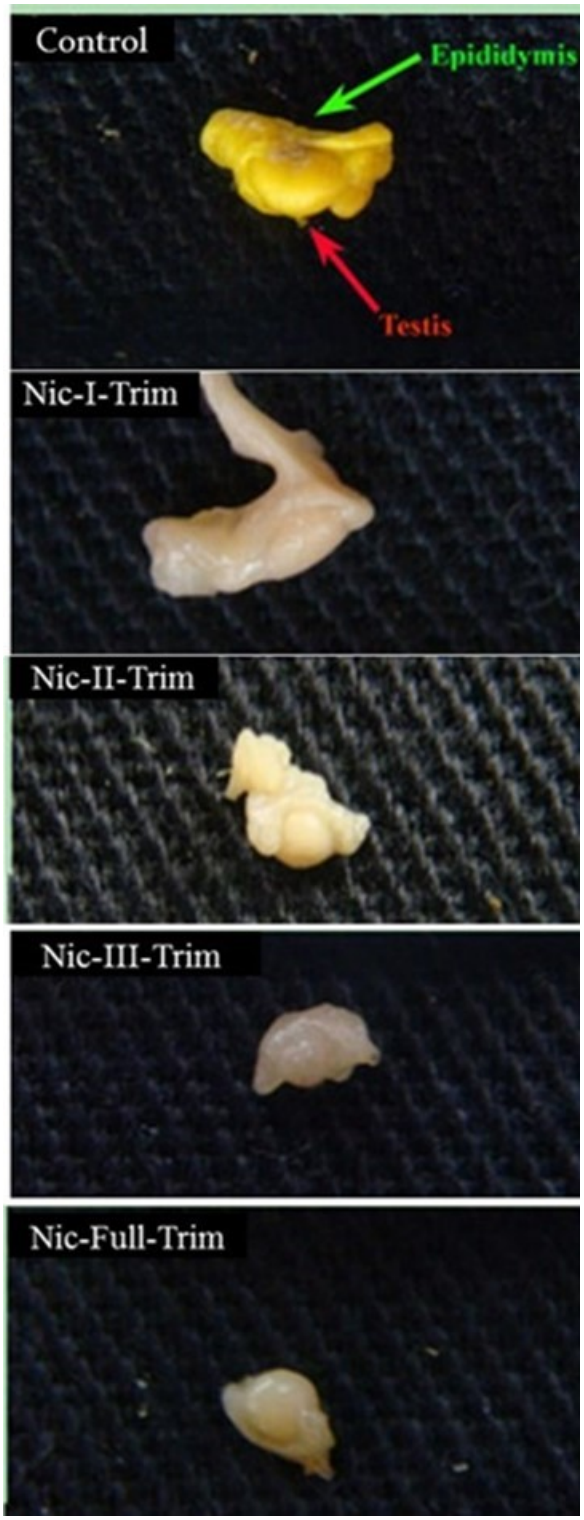
The testis were dissected out from the pups under over dose of anesthesia and fixed in Bouin's fixative and 4% of para formaldehyde. Testes were subjected to gross measurements. The tissues were processed for paraffin sectioning and stained with haematoxylin & eosin and Masson's trichrome (Collagen deposition). The stained section were observed under Nikon bright-field microscopic (Nikon Corporation, Japan) and immuno-histochemical staining to determine the extent of cellular damage through apoptosis using Caspase 3 and NFκb anti-bodies. Conventional stereological principles and accepted morphometric procedures as outlined by Elias and Hyde (1980), for morphometry in general was used in the present study to obtain quantitative information.

Results

Gross measurement of the testis showed decline in testicular size in all the nicotine treated groups (Plate 1).*H & E staining*: The testes from Nic-I-Trim and Nic-II-Trim groups showed degenerative changes in the seminiferous tubules.

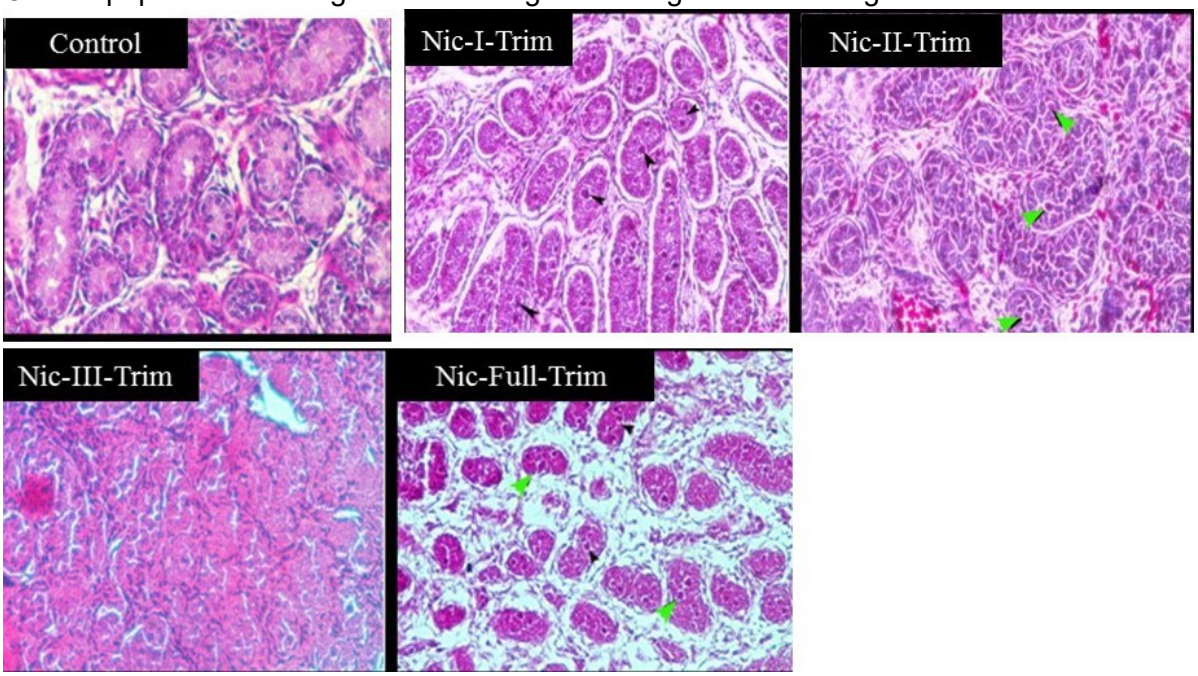
In Nic-III-Trim group rat testis showed reduced seminiferous tubular diameter and decrease in germ cell number. The severity of the damage was more obvious in Nic-Full-Trim group rat testis with very much reduced tubular diameter and increased degenerating cells (Plate 2).

Plate 1: Morphology of testis taken from different experimental groups. Note the reduction in size of the testis taken from nicotine treated rats.



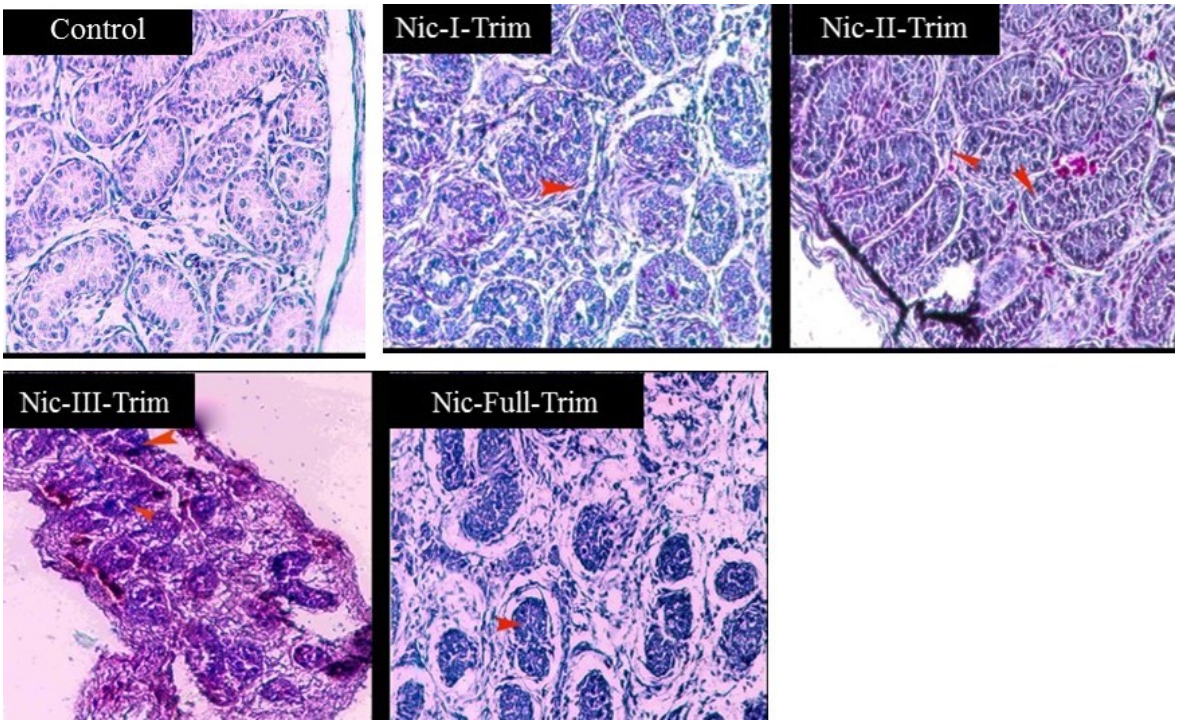
IJAS 2015, 5(2): 49-56

Plate 2: Photomicrograph of neonatal rat testis images from control and nicotine treated. Control pup testis showing normal histo-genesis of germ cells along seminiferous tubules.



Nicotine treated rat pup testis showed degenerative changes (black arrow head). Increased damages were observed in neonatal testis from maternal rat treated with nicotine for whole trimesters (Nic-Full-Trim group). Note few abnormal cells (giant cells) in the lumen of the tubules (green arrow). H & E 20x magnifications.

Plate 3: Photomicrograph of neonatal rat testis images from control and nicotine treated.



Increased collagen depositions were seen in nicotine treated rat pup testis (red arrow head) than the control. Collagen deposition was more pup's testis which were treated with nicotine for whole trimesters (Nic-Full-Trim group). Masson's trichrome staining 20x magnification.

Plate: 4: Immuno-localization of Caspase 3 in neonatal testis

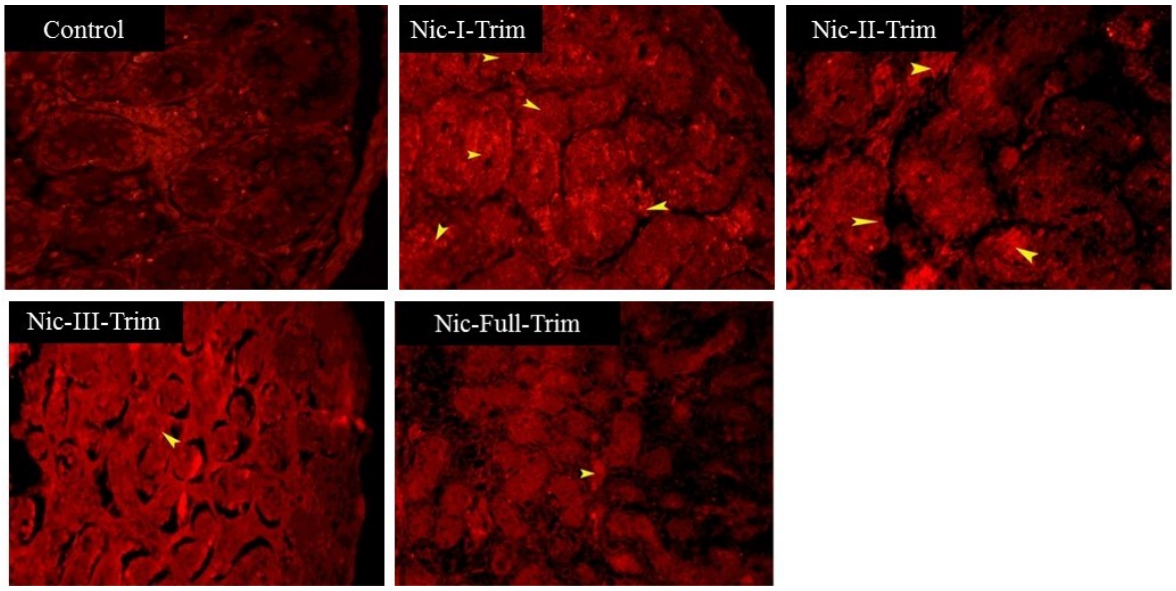


Image indicates increased or up-regulated Caspase 3 expression (arrow head) in the nicotine exposed pups testis when compare to control. Note expression was also merger in Nic-Full-Trim testis probably reduction in germ cells.

Plate 5: Immuno-localization of NFκB in neonatal testis.

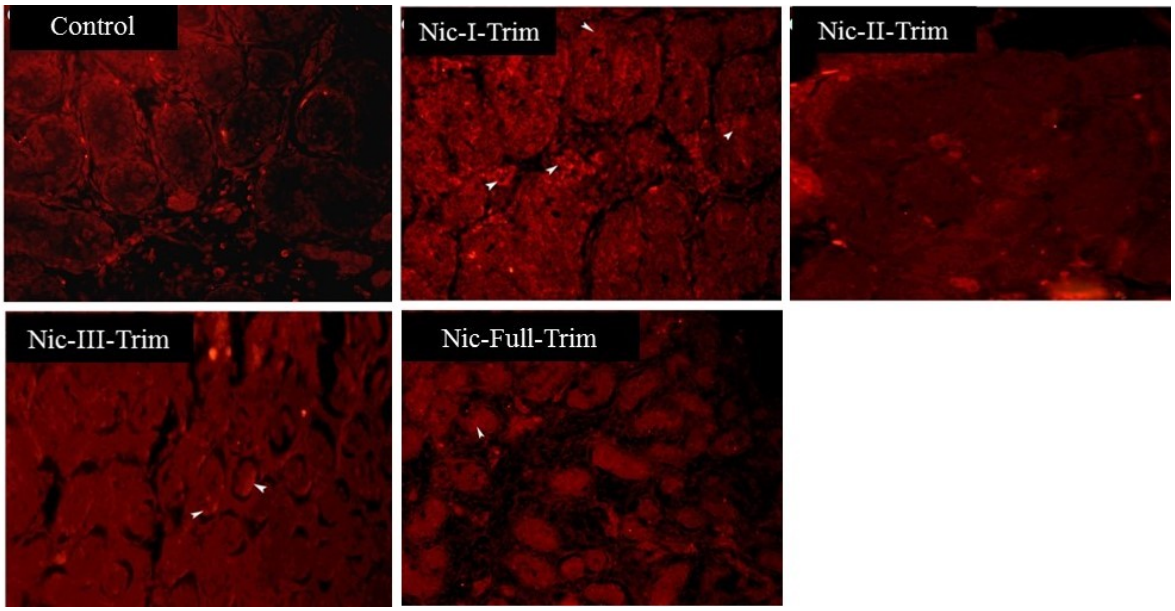
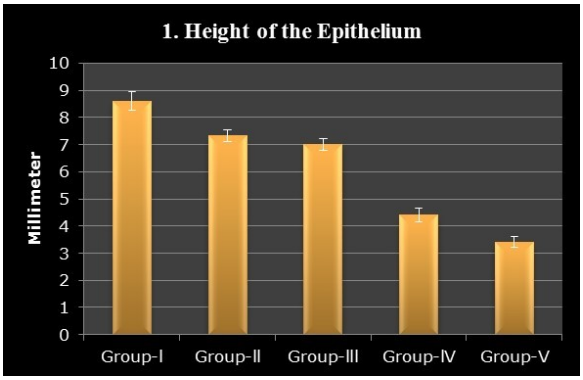
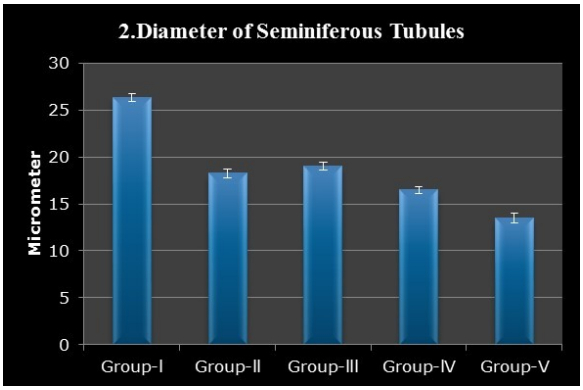
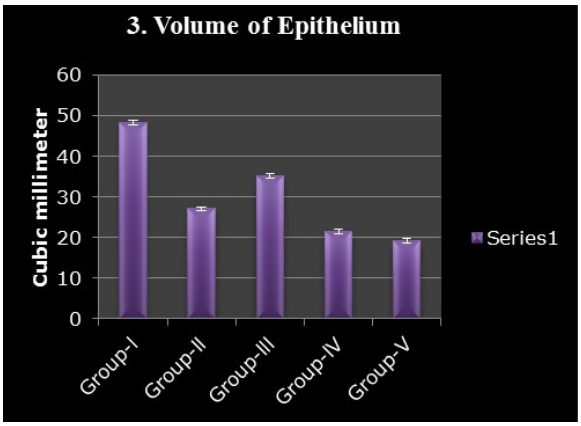


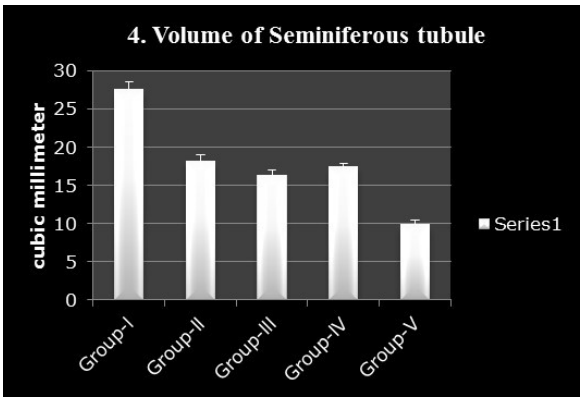
Image indicates increased or up-regulated NFκB expression (arrow head) in the nicotine exposed pups testis when compare to control. Note expression was more in Nic-I-Trim testis than the Nic-Full-Trim, may be due to the impaired germ cell loss or proliferation.

Masson's Trichrome Staining: All the nicotine treated groups showed relatively increased collagen deposition when compare to control. However, collagen deposition was markedly increased in Nic-Full-Trim group rat testis indicating atrophic changes and cellular depletion (Plate 3). Caspase 3 and NFκb anti-body staining indicate apoptotic changes in Nic-III-Trim and Nic-Full-Trim rat testes (Plate 4 & 5) *Histomorphometry:* Histo-morphometrical analysis of testis showed decrease in the height of the germinal epithelium, diameter of the seminiferous tubules, volume of the seminiferous tubules, and volume of the epithelium whereas, the connective tissue volume proportion was increased in nicotine treated rat testis (Graph 1-5).



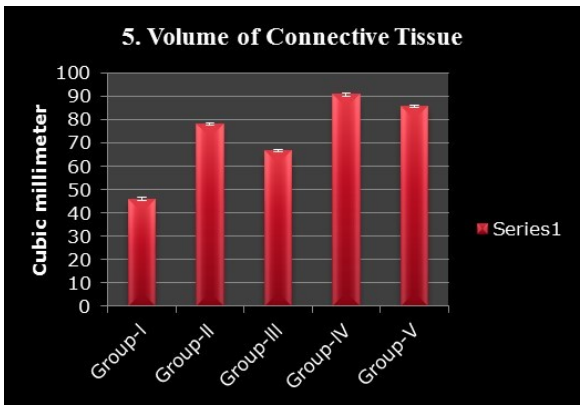






Discussion

Smoking is a major health hazard. The adverse effects of prenatal cigarette smoke exposure on human reproductive outcomes are a major scientific and public health concern. The human related scientific literature about the long-term effects of nicotine use during pregnancy is lacking, but there is substantial evidence from animal studies showing that fetal and neonatal nicotine exposure leads to widespread adverse postnatal health consequences. In our study maternal nicotine exposure during gestation caused defective gonad development which may lead to delayed puberty and reduced fertility in later life.



Developmental exposure to cigarette smoke is associated with numerous negative reproductive outcomes, ranging from alterations in germ cell morphology to an increased morbidity and mortality of offspring (DiFranza and Lew, 1995).

Histological observation from the present study revealed distorted seminiferous tubular wall and cellular degenerative changes in 1st and 2nd weeks of nicotine treatment. In 3rd week of gestation (Nic-III-Trim) showed reduced tubular size and reduced germ cells population which was also supported by histo-morphometric observations. Indeed, nicotine administration or exposure to cigarette smoke inhalation to rats and hamsters results in testicular degeneration (Viczian, 1968). In full term nicotine treated testis (Nic-Full-Trim) showed reduced testicular size and increased germ cell degeneration, degenera-ting cells in the tubules, increased interstitial space etc. Apart from these factors, the possible explanation for the pathological changes may

be due to direct effect of nicotine on the fetus by its metabolite cotine, which can inhibit rat testis androgen biosynthesis (Fowler *et al.*, 2008).There are evidences stating that nicotine freely crosses the placenta and has been found in the amniotic fluid and umbilical cord of neonates (Luck *et al.*, 1985). Intrauterine exposure to nicotine also results in decreases in birth weight (Tizabi *et al.*, 2000) indicating poor transportation of nutrients from maternal to developing fetus which could affect the general organ growth in late trimesters.

This was obvious testicular weight Nic-III-Tim and Nic-Full-Trim. Collagen deposition was markedly increased in Nic-Full-Trim group rat testis. The collagen was deposited in the interstitial space as a result of tubular atrophy and in the lumen as a result of cellular depletion.

The germ cell degeneration by nicotine treatment may be because of the low intra-testicular concentrations of testosterone, as high level of testosterone in testis is essential for the normal spermatogenesis as well as for the maintenance of the structural morphology and the normal physiology of seminiferous tubule (Sharpe *et al.*, 1992). The testosterone is required for the attachment of different generations of germ cells in seminiferous tubules, and therefore, the low level of intra testicular testosterone may lead to the detachment of germ cells from the seminiferous epithelium and may initiate germ cell death (Blanco-Rodriguez and Martinez-Garcia, 1988). Besides, nicotine and its metabolite cotinine easily enter into the blood circulation of testis and can have a direct cytotoxic effect by damaging DNA (Sepaniak *et al.*, 2006).

Programmed cell death, also known as apoptosis, is required for normal spermatogenesis in mammals and is believed to ensure cellular homeostasis and maintain the fine balance between germ cells and Sertoli cells. The proper regulation of the caspase cascade plays an important role in sperm differentiation and testicular maturity. However, caspases have been implicated in the pathogenesis of multiple andrological pathologies such as impaired spermato genesis, decreased sperm motility, increased levels of sperm DNA fragmentation and in toxicity. Increased Caspase-3 and NFκB expression in nicotine treated groups indicate activation of apoptotic pathway in germ cells. Nicotine and its metabolite cotine might have caused toxicity and initiated the apoptotic pathway, which is evidenced by increased activity of Caspase-3 and NfkB activity.

Thus indicate that Nic-III-Trim and Nic-Full-Trim group's male progeny testis were severally affected by nicotine exposure and probably would show impaired fertility at adulthood. Findings show that intra-uterine tobacco smoke increased the risk of oligozoospermia in offspring.

References

Ananth CV, Smulian JC, Vintzileos AM (1999) Incidence of placental abruption in relation to cigarette smoking and hypertensive disorders during pregnancy: a meta-analysis of observational studies. *Obstet. Gynecol*, 93: 622–628.

Benowitz NL, Porchet H, Sheiner L, Jacob P (1988) Nicotine absorption and cardiovascular effects with smokeless tobacco use: Comparison with cigarettes and nicotine gum. *Clinic Pharmacol Therapeut*, 44: 23–28.

Blanco-Rodriguez J, Martinez-Garcia C (1988) Apoptosis precedes detachment of germ cells from the seminiferous epithelium after hormonal suppression by short-term oestradiol treatment of rats. *Int J Androl,* 21: 109–115.

Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) guidelines for laboratory animal facility. Committee for the Purpose of Control and Supervision on Experiments on Animals (2003). *Ind J Pharmacol* 335:257–74.

Dempsey DA, Benowitz NL (2001) Risks and benefits of nicotine to aid smoking cessation in pregnancy. *Drug Saf,* 24: 277-322.

DiFranza JR, Lew RA (1995) Effect of maternal cigarette smoking on pregnancy complications and sudden infant death syndrome. *J Fam Pract,* 40: 385-94.

Elias H, Hyde H (1980) An elementary introduction to stereology. *Am J Anat*, 159: 411 – 446.

Fantuzzi G, Aggazzotti G, Righi E, Facchinetti F, Bertucci E, Kanitz S, *et al.* (2007). Preterm delivery and exposure to active and passive smoking during pregnancy: a case-control study from Italy. Paediatr. *Perinat. Epidemiol,* 21: 194–200.

Fowler PA, Bhattacharya S, Gromoll J, Monterio A, O'Shaughnessy PJ (2009) Maternal Smoking and Developmental Changes in Luteinizing Hormone (LH) and the LH Receptor in the Fetal Testis. *J Clinic Endocrinol Metab,* 94: 4688-4695

George L, Granath F, Johansson AL, Anneren G, Cnattingius S (2006). Environmental tobacco smoke and risk of spontaneous abortion. *Epidemiology*,17: 500–505.

Hammoud AO, Bujold E, Sorokin Y, Schild C, Krapp M, Baumann P (2005). Smoking in pregnancy revisited: Findings from a large population-based study. *Am J Obstet Gynecol,* 192: 1856– 1862

Hogberg L, Cnattingius S (2007). The influence of maternal smoking habits on the risk of subsequent stillbirth: is there a causal relation? *BJOG*, 114: 699–704.

Hung TH, Hsieh CC, Hsu JJ, Chiu TH, Lo L M, Hsieh TT (2007). Risk factors for placenta previa in an Asian population. *Int. J. Gynaecol. Obstet,* 97: 26–30.

Jaddoe VW, Troe EJ, Hofman A, Mackenbach JP, Moll HA, Steegers EA, Witteman JC (2008). Active and passive maternal smoking during pregnancy and the risks of low birthweight and preterm birth: the Generation R Study. *Paediatr. Perinat. Epidemiol,* 22:162–171.

Kulikauskas V, Blaustein D, Ablin RJ (1985) Cigarette smoking and its possible effects on sperm. *Fertil Steril*, 44: 526-8.

Lindley AA, Becker S, Gray RH, Herman AA (2000). Effect of continuing or stopping smoking during pregnancy on infant birth weight, crown-heel length, head circumference, ponderal index, and brain:body weight ratio. *Am J Epidemiol,* 152: 219–225.

Luck W, Nau H (1985). Nicotine and cotinine concentrations in serum and urine of infants exposed via passive smoking or milk from smoking mothers. *J Pediatr*, 107: 816–820.

Mitchell EA, Milerad J (2006). Smoking and the sudden infant death syndrome. *Rev. Environ. Health,* 21: 81–103.

Olfert ED, Cross BM, McWilliam AA. Guide to the care and use of experimental animals. Volume 1. 2nd ed. Canadian Council for Animal Care; 1993.

Pickett KE, Wakschlag LS, Dai L, Leventhal BL (2003). Fluctuations of maternal smoking during pregnancy. *Obstet. Gynecol*, 101: 140–147.

Rogers JM, Abbott BD (2003) Screening for Developmental Toxicity of Tobacco Smoke Constituents. *Toxicol Sci,* 75: 227-228

Sarasin FP, Hanusa BH, Perneger T, Louis-Simonet M, Rajeswaran A, Kapoor WN (2003) A Risk Score to Predict Arrhythmias in Patients with Unexplained Syncope. *Acad Emerg Med*, 10: 1312–1317.

Sepaniak S, Forges T, Gerard H, Foliguet B, Bene MC, Monnier- Barbarino P (2006) The influence of cigarette smoking on human sperm quality and DNA fragmentation. *Toxicology*, 223: 54–60.

Sharpe RM, Franks S (2002). Environment, lifestyle and infertility—an inter-generational issue. *Nat. Cell Biol*, 4: 33–40.

Stillman RJ, Rosenberg MJ, Sachs BP (1986). Smoking and reproduction. *Fertil. Steril,* 46: 545–566

Suresh S, Prithiviraj E, Prakash S (2009) Dose- and time-dependent effects of ethanolic extract of Mucuna pruriens Linn. seed on sexual behaviour of normal male rats. *J Ethnopharmacol,* 22:497–501.

Tizabi Y, Perry DC (2000) Prenatal nicotine exposure is associated with an increase in [125I] epibatidine binding in discrete cortical regions in rats. *Pharmacol Biochem Behav*, 67:319-23.

Viczián M (1968) Experiences with sperm examination of smokers. *Orv Hetil,* 19: 1077-9.

Weihe WH (1983) The UFAW handbook on the care and management of laboratory animals : The Laboratory rat, 309-30

International Journal of Anatomical Sciences 2014, 5(2): 57-65.

Research Article

Restorative Effects of Glycyrrhizic Acid on Neurodegeneration and Cognitive Decline in Chronic Cerebral Hypoperfusion Model of Vascular Dementia in Rats

Yogesh Kanna S, Kathiravan K, Pradeep Kumar N, Ramesh Kumar R.

Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, Tamil Nadu, India.

Key Words: Chronic cerebral hypoperfusion, Glycyrrhizic acid, novel object recognition, degeneration

Abstract: The present study is aimed to analyse the neuroprotective effect of Glycyrrhizic acid (GA) on chronic cerebral hypoperfusion induced neurodegeneration and cognitive decline. Chronic cerebral hypoperfusion (CCH) is a chronic reduction in cerebral blood flow associated with the aging and progressive neurodegenerative disorders which can precipitate profound cognitive decline. The experimental model for hypoperfusion is employed to elucidate the histopathological and behavioural impairments in rats by permanent occlusion of bilateral common carotid arteries (2-vessel occlusion-2VO). Here, we have focused on the effect of GA on the development of oligaemic stress resulted due to hypoperfusion. GA is known for its various pharmacological properties including antioxidant and anti-inflammatory effects. Rats were administered GA for 30 days following 2VO surgery at a dosage of 20mg/kg body weight intraperitoneally. After CCH the rats were tested for executive function, concurrently they were also probed for Neophilia through Novel Object recognition test and exploratory drive through Hole board test. The cell density of viable pyramidal in the CA1, CA2, CA3 and DG region of dorsal hippocampus was also counted and White matter rarefaction in the corpus callosum was also examined. The outcome of this study clearly implies that GA treatment could mitigate the pathogenesis of degeneration and protect pyramidal cell damage in various region of hippocampus and augment retention of hippocampal associated learning and memory and rats score significantly high in discrimination index, these findings helps us ascertain the neuroprotective potentials of GA against Vascular dementia.

Vascular dementia (VaD) ranks second in the most common form of dementias after Alzheimer's disease (AD) in older adults, accounting for ~20% of all dementia cases worldwide (Battistin and Cagnin, 2010). The World Health Organization estimates that 35.6 million people live with dementia, a number that is anticipated to triple by 2050 (World Health Organization, 2012; Iadecola 2013). Chronic cerebral hypoperfusion (CCH) is associated to several cerebrovascular conditions, including cerebral arteriovenous malformations, dural arteriovenous fistula, artherosclerosis, carotid stenosis/occlusion and cerebral small vessel diseases (Aliev *et al*., 2009). Chronic cerebral hypoperfusion has been associated with cognitive decline in aging, vascular dementia and Alzheimer's dementia. Moreover, the pattern of cerebral blood flow in mild cognitive impairment has

Correspondence to: Ramesh Kumar R, Department of Anatomy, Dr. Arcot Lakshmanasamy Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamil Nadu, India

Email: rameshanatomy@yahoo.co.in

Accepted: 30-Sep-2014

emerged as a predictive marker for the progression into Alzheimer's dementia.

India could be considered as the capital of vascular dementia, very much like diabetes and heart ailments. One common feature is all these diseases would eventually burden the vascular tree of the brain thus, causing CCH (Alladi *et al*., 2006). The reconstruction of a pathological condition in animal models is a suitable approach to the unraveling of causal relationships. (Farkas *et al* 2007). Unlike other models of stroke, chronic hypoperfusion has a brief ischemic and broad oligaemic phase, this oligaemic phase, is a recuperative process. This causes lacunar infarcts in cortex, subcortical areas, white matter (Wakita *et al*., 2002).It is believed that the present model Chronic cerebral hypoperfusion (CCH) is indispensable to model Sub cortical Ischemic Vascular Dementia (SIVD).

Permanent BCCAo induced chronic cerebral hypoperfusion in rats has shown indications towards severe axonal damage accompanied by white matter rarefaction and demyelination. (Wakita *et al*., 2002) Damaged axons are clearly seen to be swollen and increased in granularity. Immunohistochemistry has demonstrated all this and further that there is considerable loss of oligodendroglia. Cell death pertaining to both neurons and the oligodendroglia follows predominantly the apoptotic pathway. In novel mice models of chronic hypoperfusion induced by BCCAo stenosis considerable cerebral blood flow recovery was seen by 14th day of the surgery. Pronounced White matter lesions in corpus callosum adjacent to lateral ventricle were appreciated as early as 14 days. Mild white matter rarefaction was also seen in anterior commissure and optic tract at the same time microglial and astroglial activation was also noted in white matter. Diffuse grey matter lesion was also observed (Shibata *et al*., 2004).Several experimental studies have been carried out to combat the deleterious effect of cerebral ischemia and to promote a better therapeutic module which can benefit the brain function by protecting the neurons against the ischemic damage. Natural products, especially medicinal plants, could be an ideal source to develop safe and effective agents for neuroprotection against cerebral ischemia (Kim, 2005).

Glycyrrhizic acid (a triterpenoid saponin) also known Glycyron, Glycyrrhizin, It is obtained from *Glycyrrhiza glabra* (Licorice). References to licorice date back to approximately 2500 BC on Assyrian clay tablets and Egyptian papyri. It has been used as both a food and a medicine since ancient times. The genus name, meaning 'sweet root', is attributed to the first century Greek physician Dioscorides. The herb is also popular in traditional Chinese and Ayurvedic medicines, where it is known as Adhimadhuram in Tamil, Irattimadhuram in Malayalam and Yashtimadhu in Sanskrit (Blumenthal *et al*., 2000).GA is reported to have antioxidative (Kim *et al*., 2012) and anti-inflammatory effects (Genovese 2008), it is alsot reported to possess deflocculant property, rich in IL 2 activity which boosts the immunity (Ploeger 2001). Used as a Lipid lowering agent (Visavadiya and Narasimhacharya 2006), anti-depressant (Dhingra and Sharma 2006), Cognitive enhancer (Sharifzadeh *et al*., 2008), neuroprotectant (Yu *et al*., 2008; Kim *et al* 2012).This present study is aimed to understand the effect of *GA* on the CCH induced learning impairment and memory disorder and the neurodegenerative consequences.

Materials and Methods

Animals

Eighteen healthy adult Male Sprague Dawley rats weighing about 250- 300 grams were used for this study. They were maintained in an optimum environment of constant temperature (21◦C), humidity and 12 hours of day and night cycle. Animals were fed with standard food pellets and water ad libitum. The experiments were conducted in accordance with the standard guidelines of the Institutional Animal Ethical Committee (IAEC).

Chronic cerebral hypoperfusion (CCH) Model

CCH was performed using the Two-vessel occlusion method (2VO) i.e. Bilateral common carotid artery, (Farkas *et al*., 2007) briefly the animals were anesthetized with ketamine and xylazine (80 mg and 10 mg/kg body weight, intraperitoneally). The surgical procedure involves permanent ligation of the bilateral common carotid artery. For sham group the above mentioned surgical procedures were performed, except bilateral common carotid artery occlusion. This study includes three experimental groups i.e. (i) Sham, (ii) CCH and (iii) Post treatment of Glycyrrhizic acid *(GA)* for 30 days following by CCH. After surgical procedures rats were maintained for a week under proper post-operative care.

Glycyrrhizic acid procurement and Administration

Glycyrrhizic acid was procured from Sigma,USA in powder form and same was dissolved in saline and administered at a dosage of 20 mg/ Kg body weight intraperitoneally for 30 days.

Behavioural studies

Novel Object Recognition test

This is used to assess the neophilic tendencies of rats (Sarti *et al*., 2002). The arena where the Novel object recognition test (NOR) was conducted in the open field arena dimension (100 cm X 100 cm X 45 cm) The objects to be discriminated were made of plastic, colored and were in three different shapes: cubes of 6 cm side, hemispheres 8cm diameter, and cylinders of 8 cm height. The day before testing the rats were allowed to explore the box for 5 min without objects (acclimatization phase). The test is a bipartite process where in the beginning rats are allowed to explore two identical objects which are present in two opposite corners of the arena, and the amount of time taken by each rat to explore of both the objects was recorded. Snout at a distance less than 2 cm from the object and/or touching it with the snout is considered as exploring. In the second part of the process, one of the objects presented in the first trial was replaced by a new object and the rats were left in the box for 5 min. The time spent for the exploration of the familiar (F) and the new (N) object was recorded separately. In normal rats the time spent to explore a new object is significantly higher than that spent to explore a familiar one. A discrimination index was arrived at using (N - F/N + F) to compare different groups. Care was taken to avoid place preference and olfactory stimuli by randomly changing the role (familiar and new object) and the position of the two objects during the second trial and cleaning them carefully.

Hole board test

Hole board (HB) test is used to assess exploratory nature in rodents. Rats were placed in the box and the incidence of head dips into the holes and total number of squares entered was recorded by an experimenter for a period of 5 min. Head dips were recorded as the rat places its head into the holes to a minimum depth such that the ears were in level with the floor of the apparatus. At the end of the trial, the rat was immediately returned to the home cage. Between each trial, the floor of the apparatus was cleaned with 70% alcohol solution (File and Wardill 1975).

Histology and Histomorphometry

Cresyl fast violet staining for quantification of viable neurons:

At the end of post-operative experimental trials the rats were euthanized with Ketamine Hydrochloride 160mg/Kg body weight and transcardially perfused with 4% paraformaldehyde in phosphate buffered saline. The animals were decapitated and brains were removed. The fore brain was processed and embedded in paraffin wax and the tissue blocks were sectioned in to 7 micron thick sections using a rotary microtome (Weswox, India). The sections obtained were stained using Cresyl fast violet (CFV) and. Density of viable neuronal population of all hippocampal subregions within the 48400 μm^2 of bilateral dorsal

hippocampus was counted at defined coronal level in blinded fashion with reticule incorporated eyepiece at a magnification of 400X using a light microscope. A quantitative estimation of cell damage was made by direct visual counting of apparently normal neurons in the bilateral CA1 area of all the three animal groups within the reticule area. Cells showing dark cytoplasm and shrinkage were not counted. (Ramesh Kumar *et al*., 2012).

Luxol fast blue staining for white matter fibre density

Brain sections at the level of dorsal hippocampus (Bregma -3.00 to -3.50) were stained with Luxol fast blue. Corpus callosum at this level was photomicrographed at 400x magnification. These images were then analysed offline using, Image J software (NIH, USA). In brief, the images were first converted to grey scale image using the image<type options in the software. The image was measured using the measure tool to get the total area. Then the image was adjusted with auto-threshold (Renyi Entropy method) to clearly delineate the areas which were not stained and measured yet again to get percentage of infarction. The value thus obtained is divided by the original area measured earlier to get the percentage of myelination.

Statistical analysis

All data were expressed as mean ± standard error mean (SEM) and the statistical analysis of the results was performed by one-way analysis of variance (ANOVA) followed by Tukey's test using Graph Pad Prism 5. p values ≤ 0.05 was considered significant.

Observations

The effect of treatment of *GA* on the Chronic cerebral hypoperfusive rats was well documented in this study through their behaviour in NOR and HB task and the histological assessments.

Novel object recognition Test

On day 30 after BCCAo the Discriminative index of lesion group (0.1917±0.0305, Mean ± SEM) was significantly lower than sham group. On the other hand of treated group (0.5047±0.0268) was found to be significantly higher than lesion group (Fig 1A). Neophilia is a complex behaviour to understand, however this pathology seems to be complex because it believed that many cognitive substrates play major roles and thus when the rats experience anhedonia, they do not venture in NOR test, it is a must to mention that GA helps in the improvement of the condition and hence the rats exhibit neophilia.

Hole board test

The Exploratory drive was measured in terms of head dips during 5 minutes period on day 30 after BCCAo was significantly lower in lesion group (25.80±1.420) when compared to the sham group rats and the treated group (36.10±1.933) made significant increase in head dips than lesion group rats (Fig. 1B). This makes it clear that the exploratory drive follows a gradual diminishing trend, however the treatment seems to rescue.

Histomorphological investigation of grey and white matter.

Grey matter degeneration and consequent salvage by GA

Chronic cerebral hypoperfusion (CCH) is a very peculiar and rather different form of cerebrovascular ailment where the distortion in cytoarchitecture doesn't always follow a similar trend as that of the focal cerebral ischemia. Cresyl fast violet being the vital stain used in neuro-histology for demonstrating viable neurons was employed to determine the status of the neurons in lesion and treatment. Histological investigations of sham rat brains were made and it revealed pristine looking neurons with centrally placed pale round profiled nucleus and dark nucleolus , thin rim of cytoplasm stained bluish violet, prominent nissl substance which was stained in a similar fashion, indicating nuclear health and robustness and in no case there was any hint

of chromatolyis as well. The aforementioned features were regarded as inclusion criteria for selection of viability in lesion and treated tissues for quantifying spared neurons (Fig. 3 and 4).

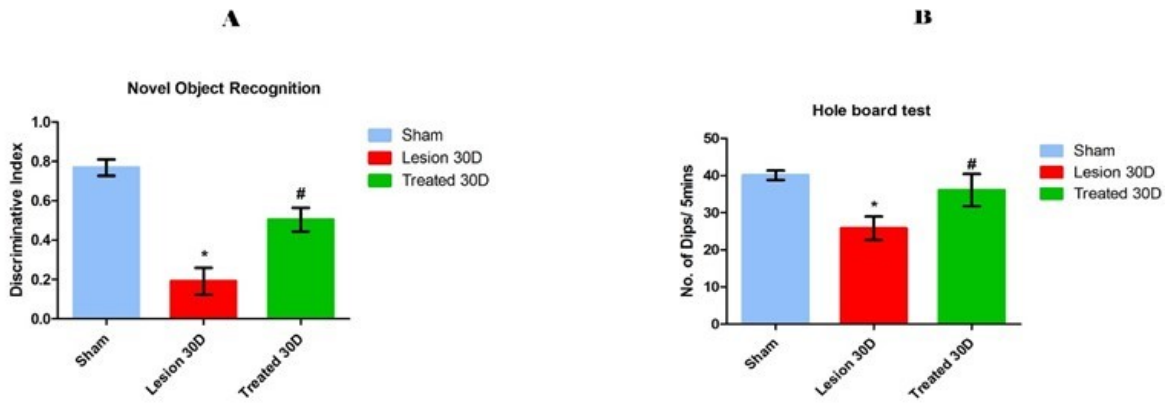
The count of normal pyramidal neurons within the 48400µm^2 of defined CA1 region of bilateral hippocampus in the sham group rats was 59.39±1.263 (Mean ± SE). The cell count in the lesion group (24.89±0.8778) was significantly reduced when compared with sham group rats and the treated group rats (40.06±1.230) show a significant increase in number than the lesion group (Fig.3A). CA2 region of bilateral hippocampus in the sham group rats was 41.72±1.631, the cell count in the lesion group (18.78±0.9273) was significantly reduced when compared with sham group rat and in the treated group (30.39±1.401) and CA2 pyramidal neurons of treated group rats were significantly higher than lesion group (Fig.3B). CA3 region of bilateral hippocampus in the sham group rats was (26.28±1.123), the cell count in the lesion group (16.89±0.8161) was significantly reduced when compared with sham group rats. However, the treated group (22.72±0.604) was significantly higher than lesion group (Fig.3C).

DG region of hippocampus in the sham group rats was 89.44±2.916. The DG cell count in the lesion group (36.06±2.0070) was significantly reduced when compared with sham group rats but in the treated group (58.78±2.337) it was found to be significantly higher in number than that of the lesion group (Fig.3D).

White matter derangement and concurrent restoration by GA

Myelin staining reveals aqua blue coloured thick intense staining with no unstained spaces and vacuolation in sham rat brain tissue. However, in lesion it was evident that there was pericellular spacing, rarefaction of myelin bundles and was found to be reduced staining intensity in the tissue, at times looked oedematous as well. Quantification of fibre density using the Renyi entropy protocol through ImageJ was performed to substantiate the potentials of white matter salvaging activity of GA. (Fig. 4). Myelin density of the sham group rat was 93.97±0.5729, lesion group (32.07±0.5093) was severely affected than sham group; the treated group (63.72±0.5304) rats showed significant restoration in fibre density in corpus callosum, when compared with lesion group (Fig. 4 a-c).

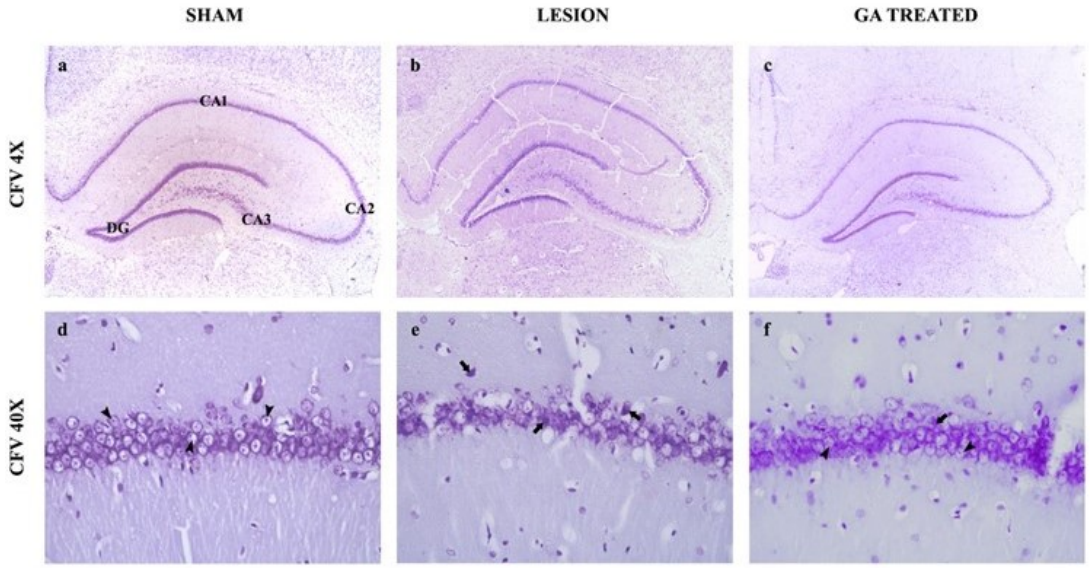
Fig. 1 Novelty seeking behaviour and Non spatial memory assessment using Novel object recognition test (A) and Exploratory behaviour assessment using Hole board test (B)



Effects of GA on chronic cerebral hypoperfusion induced neophilia and other higher cognitive deficits in rats. (A) Discriminative indices of rats after drug administration post lesioning. (B) No of head dips made during the hole board trial. Data represents Mean ± SEM of various groups which are analysed by ANOVA and compared through Turkey's test where n=6, *p < 0.05 vs sham ; # p < 0.05 vs lesion.

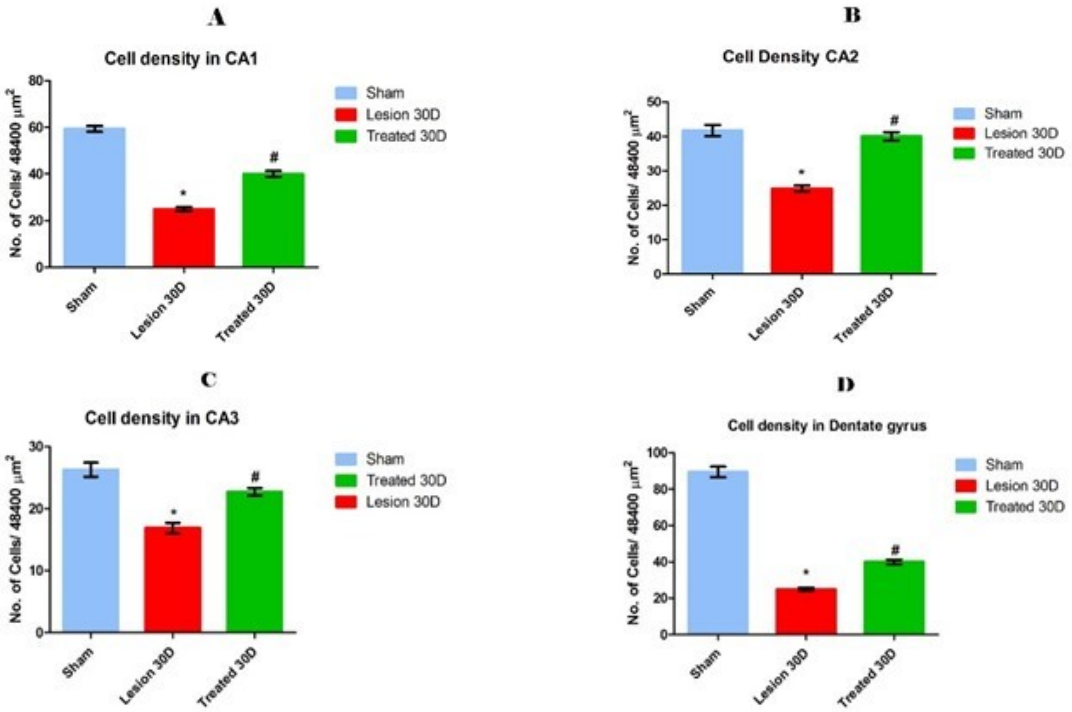
IJAS 2015, 5(2): 57-65

Fig. 2 Histological appearance of hippocampus in animals of experimental groups
Effects of GA on chronic cerebral hypoperfusion induced neuropathological changes via CFV; 40x magnification of



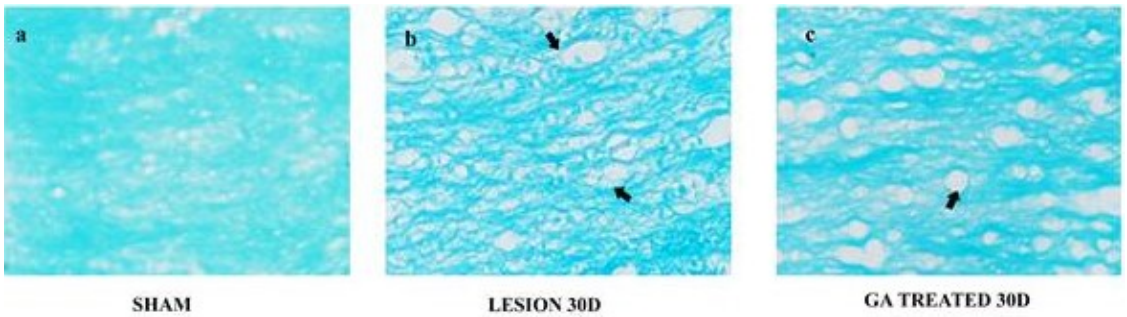
CA1 after 30 days follow up period. (2a-c) depicts 4x images of hippocampus representing the three groups and the various subfields viz. CA1, CA2, CA3, (CA- Cornu Ammonis) DG (DG-Dentate gyrus). (2d-f) 40x images illustrating viable cells (black pointed arrow) and lesioned pyknotic and shrivelled cells (black bold arrow) treated tissue exhibited coexistence of both populations

Fig.3. Effects of GA on chronic cerebral hypoperfusion induced neuropathological changes via histomorphometry quantification.

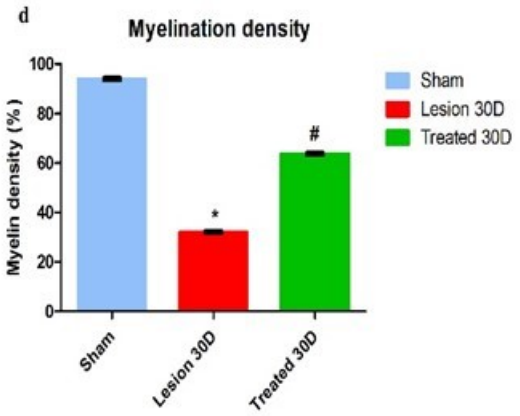


A to D histograms depicting the mean no: of viable cells from the various subfields of hippocampus in the previously mentioned CFV stained sections Data represents Mean ± SEM of various groups which are analysed by ANOVA and compared through Turkey's test where n=6, *p < 0.05 vs sham ; # p < 0.05 vs lesion.

Fig. 4 Histological appearance of Corpus callosum (myelin density) in animals of experimental groups



Effects of GA on chronic cerebral hypoperfusion induced myelin derangements like vacuolation, rarefaction 3(a-c) black bold arrows show regions of demyelinated, vacuolated and rarefied white matter, which is maximum in lesion panel and d) The histogram also substantiates the same through image-J quantification. Data represents Mean ± SEM of various groups which are analysed by ANOVA and compared through Turkey's test where n=6, *p < 0.05 vs sham; # p < 0.05 vs lesion.



Discussion

Permanent bilateral common carotid artery occlusion as a model for chronic cerebral hypoperfusion and vascular dementia affects the brain severely with its chronic nature of insult (Wakita *et al*., 2002). Cerebral blood flow (CBF) pattern is quite different from that of the transient focal ischemic events, the CBF undergoes a dynamic change, initially, it drastically falls down (ischemic phase) and then the vertebro-basilar tree takes over the job of recuperating the CBF (oligaemic phase) this lasts for 8 weeks (Farkas *et al*., 2007).

In our study Glycyrrhizic acid administration has been mainly a protective and preventive type of module for grey and white matter derangement in the 30 day study period. GA was administered through the entire period which results in alleviation of the pathology of chronic cerebral hypoperfusion. Hence, the effect of Glycyrrhizic acid has been primarily to protect the cells from oligaemic burden and shows promising inclination. Histologically it has been inferred from our work that the model has a global diffuse effect which was spanning to 30 days. The analysis of lesion group has revealed a range of deleterious changes in various areas includes striatum, hippocampal CA1, CA2, CA3 and DG. CA1 region of hippocampus is known to be the most vulnerable subgroup of cells (Niizuma *et al*., 2008) and it serves as an important cognitive substrate in learning and memory (Zhao *et al*., 2014). On the other hand, lesioning of CA2 cells causes temporal order processing deficit in Novel object recognition like paradigms. To put it simply, rats bearing CA2 lesion wouldn't be able distinguish the novel from familiar object and they cannot process. when this novel object was introduced in the maze, this makes them explore both the objects with impaired level of curiosity, thus scoring low in discriminative index (Caruana et al., 2012). CA3 cells are specialized in place and object,

place and odour association in the learning process, lesioning the CA3 greatly impairs the associative component in learning. Finally, DG as we all know is the main input for the trisynaptic network (CA3 - CA2-CA1) in hippocampus (Ji et al., 2008).

Cell density in hippocampus in treated group clearly hints towards an anti-oxidant property of the drug being involved in preventing the cells from damage. GA has been shown to possess inhibiting effect on 11-β hydroxysteroid dehydrogenase (11 β HSD) enzyme activity thus exerting an anti-inflammatory effect (Ploeger 2001). This implies that it has the property of reducing inflammatory response, countering one important cascade of events that lead to oligaemic neuronal death. *GA* is reported to possess strong antioxidant and free radical scavenging activity (Ploeger 2001; Visavadiya *et al*., 2009).

White matter rarefaction was also observed to be significant in lesion rats. In the lesion groups, it was observed that the corpus callosum was left with only half as much as the original degree of fibre density (Fig 4). Chronic cerebral hypoperfusion leads to constant production of ROS and this in turn results in lipid peroxidation (Farkas *et al*., 2007). This could be an explanation to the severe white matter rarefaction that has been observed (Shibata *et al*., 2004). However, GA appeared to have a significant ameliorating effect on white matter damage induced by oxidative stress (Fig 4) which was seen in our study.

Loss of pyramidal neurons in the hippocampus and the behavioural changes have also been in congruence with the cell loss, which was documented with Novel Object Task (Sarti *et al*., 2002). The vast amount of cell death observed could be the result of a variety of cell death cascades, such as those brought about by inflammation and apoptosis, in response to the chronic ischemic insult (Bennett *et al*., 1998; Harukuni and Bhardwaj 2006). This change in discriminative index should be analysed from a striatal perspective because it plays a major role in executive function. The reduction in exploratory drive of the rats following lesion was recuperated after treatment with GA (Sarti *et al*., 2002). Treated rats showed significantly higher levels of neophilia, as compared to the lesion group. The improvement in cognitive functions after treatment with GA could be a result of restored function of NMDA receptors which are involved in excitotoxity induced by CCH.

Conclusion

In this study the effect of Glycyrrhizic acid on the rat model of chronic cerebral hypoperfusion was quite perceivable to be a fitting arsenal against the insult posed by chronic cerebral hypoperfusion. It is seen to improve the learning and memory function and also made consistent improvement in non-spatial memory domains and higher cognitive functions. Also reduced the count of neural death in various sub regions of hippocampus claiming a definitive role in mitigating chronic cerebral hypoperfusion mediated neurodegeneration and cognitive decline.

References

Alladi S, Kaul S, Meena AK, Somayajula S, Umadevi M, Reddy JM (2006) Pattern of vascular dementia in India: study of clinical features, imaging, and vascular mechanisms from a hospital dementia registry. *J Stroke Cerebrovasc Dis*, 15: 49-56.

Aliev G, Smith MA, Obrenovich ME, Jack C, Perry G (2003) Role of vascular hypoperfusion-induced oxidative stress and mitochondria failure in the pathogenesis of Alzheimer disease. *Neurotox Res*, 5: 491-504.

Battistin L, Cagnin A (2010) Vascular cognitive disorder. A biological and clinical overview. *Neurochem Res*, 35: 1933-1938.

Bennett SA, Tenniswood M, Chen JH, Davidson CM, Keyes MT, Fortin T, Pappas A (1998) Chronic cerebral hypoperfusion elicits neuronal apoptosis and behavioral impairment. *Neuro Rep,* 9: 161–166.

Bevins RA, Besheer J (2006) Object recognition in rats and mice: a one-trial non-matching-to-sample learning task to study recognition memory. *Nat Protoc*, 1: 1306-1311.

Blumenthal M, Goldberg A, Brinckmann J (2000) Herbal medicine: expanded Commission E

monographs. Austin, TX: Integrative Medicine Communications. 237.

Caruana DA, Alexander GM, Dudek SM (2012) New insights into the regulation of synaptic plasticity from an unexpected place: hippocampal area CA2. *Learning & memory*, 19: 391-400.

Dhingra D, Sharma A (2006) Antidepressant-like activity of Glycyrrhizaglabra L. in mouse models of immobility tests. *Prog Neuropsychopharmacol Biol Psychiatry*, 30: 449–454.

Farkas E, Luiten PG, Bari F (2007) Permanent, bilateral common carotid artery occlusion in the rat: a model for chronic cerebral hypoperfusion-related neurodegenerative diseases. *Brain Res Rev*, 54:162-180.

File SE, Wardill AG (1975) Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacol*, 44: 53-59.

Genovese TM (2008) Glycyrrhizin reduces secondary inflammatory process after spinal cord compression injury in mice. *Shock* 31: 367–375.

Harukuni I, Bhardwaj A (2006) Mechanisms of brain injury after global cerebral ischemia. *Neurol Clin*, 24: 1-21.

Iadecola C (2013) The pathobiology of vascular dementia. *Neuron*, 80: 844-866.

Ji J, Maren S (2008) Differential roles for hippocampal areas CA1 and CA3 in the contextual encoding and retrieval of extinguished fear. *Learning & Memory*, 15: 244-251.

Kim H (2005) Neuroprotective herbs for stroke therapy in traditional eastern Medicine. *Neurol Res*, 27: 287-301.

Kim SW, Jin Y, Shin JH, Kim ID, Lee HK, Park S, Lee JK (2012) Glycyrrhizic acid affords robust neuroprotection in the post ischemic brain via anti-inflammatory effect by inhibiting HMGB1 phosphorylation and secretion. *Neurobiol Dis*, 46: 147-156.

Kim SW, Lim CM, Lee HK, Lee JK (2011) The use of Stronger Neo-Minophagen C, a glycyrrhizin-containing preparation, in robust neuroprotection in the post ischemic brain. *Anat Cell Biol*, 44: 304-313.

Ramesh Kumar R, Kathiravan K, Muthusamy R (2012) Bacopa monniera a Potent Neuroprotector against Transient Global Cerebral Ischemia Induced Hippocampal Damage and Memory Function. *Int J Anat Sci*, 3: 26-32.

Niizuma K, Endo H, Nito C, Myer DJ, Kim GS, Chan PH (2008) The PIDDosome mediates delayed death of hippocampal CA1 neurons after transient global cerebral ischemia in rats. *PNAS*, 105: 16368-16373.

Ploeger B, Mensinga T, Sips A, Seinen W, Meulenbelt J, DeJongh J (2001) The pharmaco-kinetics of glycyrrhizic acid evaluated by physiologically based pharmacokinetic modeling. *Drug Metab Rev*, 33: 125-147.

Sarti C, Pantoni L, Bartolini L, Inzitari D (2002) Persistent impairment of gait performances and working memory after bilateral common carotid artery occlusion in the adult wistar rat. *Behav. Brain Res*, 136: 13–20.

Sharifzadeh M (2008) A time course analysis of systemic administration of aqueous licorice extract on spatial memory retention in rats. *Planta Med*, 74: 485–90.

Shibata M, Ohtani R, Ihara M, Tomimoto H (2004) White matter lesions and glial activation in a novel mouse model of chronic cerebral hypoperfusion. *Stroke*, 35: 2598-2603.

Visavadiya NP, Narasimhacharya AV, (2006) Hypocholesterolaemic and antioxidant effects of Glycyrrhizaglabra (Linn) in rats. *Mol Nutr Food Res*, 50:1080–1086.

Visavadiya NP, Soni B, Dalwadi N (2009) Evaluation of antioxidant and anti-atherogenic properties of Glycyrrhiza glabra root using in vitro models. Int J Food Sci Nutr, 60: 135-149.

Wakita H, Tomimoto H, Akiguchi I, Matsuo A, Lin JX, Ihara M, McGeer PL (2002) Axonal damage and demyelination in the white matter after chronic cerebral hypo perfusion in the rat. *Brain Res*, 924: 63-67.

International Journal of Anatomical Sciences (IJAS)
Instructions to Contributors

1. IJAS welcomes original research findings / observations on all aspects of bio-medical field which has a relevance to structure and structural changes. Articles without any reference to structure are not in the scope of the journal
2. IJAS accepts articles from authors with an assumption that they were not previously published in any form and if accepted for publication in IJAS shall not be published in any other form.
3. Papers must be submitted only through email to the address editor.ijas@gmail.com and submission in any other form will not be accepted.
4. A paper can be of maximum 20 pages in A4 paper size including illustrations, legends, acknowledgements, and references.
5. All the papers submitted to IJAS will be subjected to review by at least two referees before editorial takes a decision on the acceptance of the paper for publication. Review process of IJAS is an anonymous double-blind process.
6. IJAS is an open-access journal. Therefore, anyone can download published articles free of cost from its online portal. Authors of accepted articles have to pay Rs.1000/- or the charges as fixed by the Association of Anatomists, Tamil Nadu from time to time. These charges are towards publication cost and online uploading charges.
7. No paper reprints will be supplied to authors of accepted articles since the journal itself is an open access type. However, if authors wish to purchase hard copy of the journal, they should contact, editorial office and pay the cost as fixed by the Association of Anatomists, Tamil Nadu from time to time. Currently the rate of one single hard copy of the journal is Rs.1000/-
8. Authors must prepare their papers as per the detailed instructions available in the website. Failure to follow the instructions in any manner will result in the rejection of the submitted paper.
9. Please visit www.ijas.in for details regarding preparing papers for submission to IJAS. In case of any difficulty in accessing the online portal, contact the journal office at Department of Anatomy, Dr.A.L.M. PGIBMS, University of Madras, Taramani Campus, Chennai 600 113, India. Phone: 91-044-24547020 Email: editor.IJAS@gmail.com