1. INTRODUCTION

Nanotechnology involves the creation and development of materials, which has at least one dimension less than 100 nanometer (nm). This emerging ability to manipulate matter at the molecular level has allowed the scientists to create an array of potential commercial products. New nanomaterials are constantly being engineered, but certain ones are commonly used in clinical therapies and diagnosis including metal oxide NPs, quantum dots, fullerens and carbon nanotubes (Lin, 2006). Over 600 nano-based raw materials and intermediates are already on the commercial market, and this number will certainly increase in the future (Meili, 2006).

Nanomaterials are widely used not only in industry, but also have been extensively explored for possible applications in medicine. These manufactured nanomaterials can possess unique chemical and physical properties that cannot be predicted based on the current understanding of their behavior in larger bulk forms. They pose potential health and environmental hazard on interaction with biological systems which may lead to adverse effects. As nanotechnology has become an inevitable member in day-to-day life’s requirements, more definitive research on nanotoxicology has to be carried out in order to create suitable regulations to ensure safe usage of nanomaterials (Lin, 2006).

Ultrafine superparamagnetic iron oxide nano particles (USPIONs) with appropriate surface chemistry are promising materials which hold immense potential in a variety of biomedical applications such as magnetic resonance imaging (MRI), targeted delivery of drugs or genes, targeted destruction of tumour tissue through hyperthermia, magnetic transfections, chelation therapy and tissue engineering (Huber, 2005; Gupta and Gupta, 2005; Hautot et al., 2007). Among the various forms of USPIONs, Ferric oxide (Fe$_3$O$_4$) nanoparticles (NPs) has been preferably used in various biomedical applications, including neuroimaging and neurotherapeutics due to its potential ability to permeate blood-brain barrier (BBB). Repeated use of Fe$_3$O$_4$ NPs might disturb the iron homeostasis in brain due to iron accumulation resulting in neurodegenerative disorders. Moreover, its prolonged blood half life is a sparkling advantage over the traditional agents. Though the immediate clinical effects of Fe$_3$O$_4$ NPs as contrast agents and neurotherapeutics have been well characterized and found to be quite safe, its molecular interactions with systemic
organs raise a concern for long-term and repeated usage (Winer et al., 2011). Iron being a transition metal, can readily undergo Fenton's reaction and modulate oxidative stress in brain leading to various neurodegenerative disorders (Halliwell, 1992).

An increase in reactive oxygen species (ROS) levels leads to oxidative stress, and damage to cells. Normally, the body provides protection against ROS by producing antioxidants and detoxifying enzymes. But when the level of oxidative stress becomes too high, key cellular activities are disrupted as ROS level increases unchecked. As more and more damage is sustained, cells eventually succumb to inflammation leading to cytotoxicity. These NPs can also penetrate mitochondria and cause oxidative damage to its membranes, inducing the release of cell death factors (Nel et al., 2006; Maynord et al., 2005). Other injuries to the body, such as protein denaturation, membrane damage, DNA damage and the formation of foreign body granulomas may also occur due to the presence of nanomaterial within the cells (Ahamed et al., 2008; Lafuente et al., 2012).

Iron Oxide NPs generates more ROS than their larger counterparts because its large surface area to volume ratio presents more of the active sites per particle. As these NPs contain iron, which is a transition metal on their surfaces, they can form electron donor or acceptor active sites. For instance, experiments have shown that some of these active sites readily donate an electron to molecular oxygen to form the superoxide radical ($O_2^-$). These $O_2^-$ vigorously reacts with hydrogen to form hydrogen peroxide ($H_2O_2$), which can interact with iron ions ($Fe^{2+}$) present in the body and generate hydroxyl (·OH) radicals, a type of ROS (Zorov et al., 2006).

The iron present in these iron oxide NPs are a concern. Iron is essential for various biological processes like storage and activation of molecular oxygen, reduction of ribonucleotides and dinitrogen, activation and decomposition of peroxides and electron transport via a wide variety of electron carriers, but excess iron in the body can lead to various complications (Anderson, 2007; Taher et al., 2009).

Iron overload in central nervous system (CNS) and its impact on neurodegenerative disorders, including Hallervorden-Spatz, Parkinson disease (PD), Alzheimer’s disease (AD) has been well investigated in the past decade (Thompson et al., 2001). Furthermore, a vast number of movement disorders, including hereditary ferritinopathy, aceruloplasminemia, Kufor-Rakeb disease,
woodhouse-sakati syndrome, hemochromatosis and restless leg syndrome falls under neurodegeneration of brain iron accumulation (NBIA) (Dusek et al., 2012). Thus, the interaction of Fe$_2$O$_3$ NPs with biological systems needs to be addressed in detail as its usage is increasing in neurobiological applications. Iron oxide NPs can cause molecular level damages to brain by crossing BBB (Sharma and Sharma, 2007).

Neurotoxicity of Fe$_2$O$_3$ NPs has already been proven by various investigators. Wang et al. (2009) reported that the intranasal exposure of Fe$_2$O$_3$ NPs could induce oxidative stress, nerve damage, neurodenron and hippocampal degeneration. Additionally, Wang et al. (2007) also reported neuronal fatty degeneration in CA3 of hippocampus and trigeminus of the brain stem over inhalation of Fe$_2$O$_3$ NPs. Hautot et al. (2007) observed an elevated accumulation of Fe$_2$O$_3$ and Fe$_3$O$_4$ NPs in the basal ganglia of neuroferritinopathy patients which could cause extensive damage to brain. Decreased cell viability, diminished rate to form mature neuritis, increased cytoskeletal disruption, aberrated cell function and phenotype were observed when PC12 cells were exposed to Fe$_2$O$_3$ NPs (Pisanic et al., 2007). However, specific elucidation on the impact of Fe$_2$O$_3$ NPs on learning and memory still remains limited.

Fe$_2$O$_3$ NPs have gained the global attention in the biomedical applications; its safety is being thoroughly investigated worldwide. Apart from neurological aspects, its interaction and impact with all the systemic organs including lungs and respiratory systems are also under parallel investigation to ensure and avoid the adverse effects (Zhou et al., 2003; Zhu et al., 2008).

Oxidative stress has been shown to greatly influence locomotor behavior and locomotion has been found to be a consistently sensitive measure of toxic stress (Little and Finger, 1990; Begum et al., 2006). However, impact of oxidative stress mediated by Fe$_2$O$_3$ NPs on locomotor behavior is yet to be investigated. Moreover, iron overload in brain has been shown to cause number of neurodegenerative disorders (Thompson et al., 2001), but emphasis on locomotor behavior has not been given so far. Furthermore, iron accumulation has been reported to be a potential candidate in the early stages of neurological memory disorders such as AD (Thompson et al., 2001). However, research record focusing iron NPs mediated learning and memory impairments is limited or none.
Introduction

In this study, the hypothesis that administration of Fe$_2$O$_3$ NPs might cause learning and memory impairments was investigated, since it was shown that Fe$_2$O$_3$ NPs induce neurotoxicity (Wang et al., 2007; Wang et al., 2009). Fe$_2$O$_3$ NPs sized <50 nm were used to test the learning and memory responses in mice by intraperitoneal exposure. As the prelude, *in vitro* study of Fe$_2$O$_3$ NPs was carried out using human neuroblast IMR-32 cells of brain origin to find out the toxic effects. The cell death percentage was evaluated by MTT assay and live and dead analysis. Cell morphology and oxidative stress were also determined by measuring the activities of antioxidant enzymes. The results of our *in vitro* cytotoxicity served as the base for the rest of our investigation. Albino mice (*Mus musculus*) were intraperitoneally administered with Fe$_2$O$_3$ NPs sized <50 nm at the dose of 50 mg/kg body weight for 1 d and 30 d (each dose at an interval of 7 d). The experiments were carried out after 24 h of the last treatment. Locomotor behaviors were assessed by rota-rod motor co-ordination and spontaneous motor activity. Learning and memory was assessed by using various maze systems. The animals were then sacrificed by cervical dislocation, brain was collected and sub-brain regions were dissected out immediately. The neuronal DNA damages were analyzed by comet assay and histopathological examination was carried out to observe the tissue level changes in sub-brain regions.

General biochemical investigations including iron estimation to find out the bio-availability of Fe$_2$O$_3$ NPs and total protein estimation in sub brain tissue homogenate were carried out. Activity of acetylcholinesterase (AChE) was measured since the cholinergic system controls the motor co-ordination of the body. Oxidative damage caused by Fe$_2$O$_3$ NPs in sub-regions of brain were assessed by evaluating oxidative stress biomarkers through measuring lipid peroxidation (LPO) and the activities of antioxidant enzymes *viz.*, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx).