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KEY WORDS: Brain; Antioxidants; Oxidative Stress; Antioxidant Catalase, Reduced glutathione, *Brahmi*.

TO STUDY THEANTIOXIDANT EFFECT OF *BRAHMI* ON CATALASE AND GSH ACTIVITIY.

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ABSTRACT

The effect of *Brahmi* was studied on antioxidant activity since, impaired antioxidant activity is considered as key factor for neurodegenerative diseases. *Brahmi* is one of the reputed medicinal plant of the Ayurveda used as a nerve tonic since time immemorial. The effect of *Brahmi*was assessed on catalase and reduced glutathione(GSH). There was significant increase in the activity of catalase and reduced glutathione (GSH) on treatment with *Brahmi*. The results suggest that *Brahmi* exhibits significant antioxidant effect and helps in reducing oxidative stress. The antioxidant activity, indirectly helps in cognition and this may be facilitating memory enhancing effect of *Brahmi*. Furthermore, the present study clearly indicates that the *Brahmi*has a significant potential as a natural antioxidant agent.

INTRODUCTION

Antioxidant plays an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species. There are mainly two categories of antioxidant species: The enzymatic part is represented by free radical scavenger enzymes namely superoxide dismutase, catalase and glutathione peroxidase. The non-enzymatic half includes an large range of natural and synthetic antioxidant compounds (e.g. vitamins, thiols etc.) that have the power to inhibit oxidative stress by scavenging the extremely damaging free radicals (Halliwell, 1994,Anbarasi et al., 2006). Excess production of free radicals and reactive oxygen species (ROS), such as singlet oxygen (1O2), superoxide anion (O2), hydrogen peroxide (H2O2) and hydroxyl radicals (OH) are thought to cause damage to cells (Good et al., 1996; Gassen and Youdim, 1997; Halliwell and Gutteridge, 1999).These increased concentrations of free radicals in the body lead to various pathological conditions such as atherosclerosis, arthritis, Alzheimer's disease, cancers, ageing and neurological diseases (HalliwellB , 2007).Thus antioxidant activity provides an important shield from oxidative damage and play an important role in cognition and well- being of brain in general.

MATERIALS AND METHOD

Brahmi capsules manufactured by (The Himalaya Drug Company, Bangalore)was used for the study. The capsule was dissolved in water and used for the treatment. Stock solution of the drug was prepared in the sterile distilled water. The concentration of the drug was 150 mg per kg of body weight of the rat. Drug was administered orally. The control group of the rat was treated with the sterile distilled water in the similar manner. The drug was administered in the morning hours throughout the study period.

Animal procurement and management:

Fresh stock of the male albino rats of wistar strain (weighing 130-180 grams and 5-8 weeks of age) were used for all the experimental work. All the animals were procured were obtained from Animal House, Bombay Veterinary College, BVC Campus Road, Parel, Mumbai 012, Maharashtra, India. All the animals were weighed and their health was verified. Animals were acclimatized to the experimental environment for a minimum period of eight days prior to the commencement of the study.

All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of RamnarainRuia College, Matunga, Mumbai 019, Maharashtra, India (CPCSEA/315).

Housing:

All the animals were housed in polyurethane cages with wire mesh tops and rice husk bedding. The rice husk bedding was changed every day. Food and water was provided to the animal ad-libitum. Water was provided in an amber coloured glass bottle. A standard laboratory rat feed with balanced nutrition (crude protein 20-21%, crude fibre 4%, calcium1.2%, phosphorus 0.6%) was provided to the animals. The temperature of the animal house was maintained at 28°c (+/- 2°c). The animal house was provided with an artificial light at a sequence of 12 hrs light and 12 hrs dark cycles. Humidity of animal house was not controlled. The humidity as recorded on humitherm was between 50-77% RH during the period of experiment.

Tissue preparation

Rats were sacrificed by decapitation. Rat whole brains were rapidly removed, weighted and thoroughly washed with ice-cold saline. Then washed with cold 0.1M phosphate buffered saline(PBS) pH7.4. Resuspended tissue in 500 – 1000 µL of ice cold 0.1M PBS. Homogenize tissue with a homogenizer or pestle sitting on ice, with 10 – 15 passes. Centrifuge sample 2 – 5 minutes at 4°C in a cold microcentrifuge to remove any insoluble material. Collect supernatant and transfer to a new tube. Supernatant was used as a source of enzyme.

TheCAT activity assay was performed using spectrophotometric determination of hydrogen peroxide (H_2O_2) which form stable complex with ammonium molybdate that absorbs at 405 nm. (Goth, 1991)

The measurement of GSH uses a kinetic assay in which catalytic amounts (nmoles) of GSH cause a continuous reduction of 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) to TNB. The yellow product, 5-thio2-nitrobenzoic acid (TNB) is measured spectrophotometrically at 412 nm. The assay uses a standard curve of reduced glutathione to determine the amount of glutathione in the biological sample. The statistical analysis was performed by using student's t-test.

RESULTS AND DISCUSSION

The treatment of animals with Brahmi for 28 days resulted in significant increase in the Catalase (CAT) activity and Reduced Glutathione (GSH) activity as shown in the table 1. This increase in the antioxidant activity may be indirectly attributing the memory enhancing and cognitive enhancing properties of the Brahmi. Various in vitro and animal studies have suggested that the cognition-promoting effect of Brahmi may be partially due to its antioxidant activity. (Russo et al., 2005, Kaustubh et al., 2017).The conversion of hydrogen peroxide to oxygen and water is catalyzed by CAT. (Chelikani

et al., 2004, Neetu et al., 2012) and helps in removal of hydrogen peroxides anions and protect brain from oxidative damage. Therefore by increasing the catalase activity *Brahmi* protects brain from harmful effect of oxidative damage.Oxidative stress, inflammation and aging are important factors in brain aging which leads to concomitant cognitive decline particularly decreased memory such as recognition memory (James et al. 2008), short term recall (Gilchrist et al. 2008) and long-term memory (Park et al. 2002). The cognitive decline is result of the free radicals attacking delicate brain cells, they disrupt optimal cellular function and often cause cognitive decline. Reduced glutathione (GSH) protect against pro-oxidant stress and serves as the brain's primary antioxidant defense against prooxidant stress (Anbarasi et al., 2006). Therefore the significant increase in reduced glutathione, shows the neuroprotective effect of *Brahmi*. The accumulation of genetic defects that influence the regenerative capacity of the neural stem cells (NSC) such as telomere shortening, DNA oxidations and mitochondrial function, DNA deletions and point mutations are often results due to deficiency of dietary antioxidants, which are very crucial for genome maintenance.(Hamilton et al. 2001). This in turn affects the memory function and cognition. Supplementation with plant-derived antioxidants can reverse age-related decline in memory and cognition (Bickford et al., 2000). Therefore it can be said that Brahmi's antioxidant property is useful in cognition and memory.

Table 1: Effect of Brahmi onCatalase and Reduced Glutathione (GSH) activity.

	Control	Treated
Catalase activity	2.61 ± 0.040	7.90 ± 0.115
GSHactivity	5.28 ± 0.060	6.25 ± 0.088

The Catalase and Reduced Glutathione (GSH) activity in treated rats with the drug for 28 days were determined data represent mean ±SE, n=6 and $P \leq 0.01$.

CONCLUSION

Brahmi has potent cognitive enhancing property, with indirect mechanism on effect of the antioxidant activity. Thus, the present study advocates the immense potential of Brahmi in the promotion to boost the level of antioxidants in relation with increased antioxidant activity. The significant increase in Catalase (CAT) activity and Glutathione (GSH) activity on treated rats support its potential as a therapy in neurodegenerative pathologies and age-related cognitive decline. Therefore it could be said that brahmi through its antioxidant property protect brain from oxidative damage and facilitate its memory enhancing effect.

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