Biochemical estimation of antioxidant enzyme in cerebellum of ageing albino rats *vis a vis* effect of *Celastrus paniculatus*

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ABSTRACT

Background: The generation of reactive oxygen species and oxidative damage play a pivotal role in neuronal ageing. High rate of oxygen in take indirectly cause high rate of lipofuscin formation through lipid per oxidation, conjugation and polymerization. **Purpose:** The purpose of the present study was to determine the antioxidant effect of *Celastrus paniculatus* during ageing in cerebellum of *Rattus norvagicus*. Herbal drugs have been in use since prehistoric times for rescuing mental disorder. Even the individuals with normal cognition show age related changes as they age i.e the degenerative effects in Purkinje neurons. **Methods:** Male Wister albino rats were administered the ethanolic seed extract of Celastrus paniculatus orally at a dosage of 2g/Kg body weight for 16 days and the control animals were given same amount of distilled water. **Results:** Upregulation of Superoxide dismutase, Catalase and Glutathione peroxidasae levels indicates a significant antioxidant effect. **Conclusion:** The results of this study may be useful in relation to various brain disorders where oxidative stress plays a significant role.

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Introduction

An organism ages as a 'self-regulatory' system and its key mechanisms are linked with changes in structure, and functions of the brain[1]. The brain is metabolically one of the most active organ in the body and more susceptible to free radical attack and oxidative stress. The presence of easily peroxidizable polyunsaturated fatty acids and poor availability of antioxidant enzymes makes this organ more vulnerable to degeneration[2]. With advancing age oxidative stress increases in the brain resulting in the formation of free radicals in the body. Free radicals are highly reactive species having potential to oxidize various biological molecules including lipids, proteins, carbohydrates and nucleic acids. Free radicals activity determines the pace of lipid peroxidation resulting in membrane damage and cell death ultimately leading to the process of ageing. Thus, brain acts as a 'pace-maker' in the process of ageing. Harman[3] proposed that highly reactive derivatives of oxygen, the free radicals, are produced during normal metabolism. Despite the fact that there is continuous production of free radicals, which increase with ageing, our body possesses several defense systems that are constituted by enzymes and radical scavengers[4]. The antioxidants superoxide dismutase[5] tocopherols[6], catalase and glutathione peroxidase[7] limit the production of free radical damage to a 'tolerable level'. Genetic manipulations, transplantation of young organs and diverse drug preparations (from alternative medicine sources such as Ayurvedic, Allopathic, Unani and Sidha) have been employed from time immemorial to combat ageing. As the existing drugs do not enhance antioxidant capacity of the body we wanted to test the effect of *Celastrus paniculatus on antioxidant levels.*

Methods

Male Wister albino rats were obtained from the animal house of Chaudhary Charn Singh Agricultural University, Hisar. The animals were housed in the animal facility of the Department of Zoology, Kurukshetra University. The animals were provided standard rat feed and water *ad libitum*. Twelve-hour light and dark along with 27^{0}C temperature conditions were maintained throughout the experiments. Before starting the experiment, the animals were kept under these conditions for two weeks to acclimatization purposes. The experimental animals were divided into young control (3 months old), early age control (12 months old), aged control (20 Months old) and aged treated (20 months old) groups. Each group consisted of five animals. The study was approved by the Institutional animal ethic committee.

Drug Preparation

The seeds of *Celastrus paniculatus* were procured from and Ayurvedic medical practitioner at Kurukshetra in a single lot and sent to the Ayurvedic Department, Kurukshetra and Department of Botany, KUK for their verification and botanical identification. The seed were crushed and extracted with ethyl alcohol in 1:3 (seeds: alcohol) for 30 days and all the traces of the solvent were consequently removed. Thick brown extract obtained from the truf and used for the treatment of the rats.

Drug Schedule

Ethanolic extract of *Celastrus paniculatus* was given to aged treated group orally at a dosage of 2g/kg body weight daily at 10 A.M. for 16 days and control animals were given same amount of the distilled water.

Assays of Enzymes

The cerebellum was removed immediately after experiments and rinsed in chilled normal saline. Subsequently, homogenate was prepared and enzyme activities were measured by using standard methods and levels of different enzymes in both experimental and control groups animals carried out in cerebellar supernatants. SOD was estimated by employing the modified method.[8] The modified method of Kar and Mishra[9] was used to determine the catalase activity. For the estimation of GPx activity, method of Paglia and Valentine[10], was employed.

Results

The data for superoxide dismutase activity is given in Table 1. The three months old, young control animals showed the highest activity of SOD i.e. 14.7 ± 0.5. There was significant ($P < 0.01$) decrease in activity in early age control (8.2 ±0.3) animals. The treatment of *Celastrus paniculatus* showed the highly significant ($P < 0.01$) increase in activity of SOD in aged treated animals It was also observed SOD activity decreased during ageing.

As evident from data in Table 1 there was a decline in activity of catalase with the advancement of age. The young control rats possessed 25.0 ± 2.1 (mol/mg protein) activity of catalase. There is insignificant decline in catalase activity in early age control (22.3 ± 0.7) rats and further significant decline in age control (18.6 ± 0.9) rats. The activity of catalase diminished with age. *Celastrus paniculatus* treated rats had a non significant increase in the age treated rats. The activity of glutathione peroxidase in young control was 47.2 ± 0.8 (n mol of NADPH oxidized/min/mg protein). It has been observed that there was significant ($P < 0.01$) decrease of activity in early age control 39.6 ± 0.8 and age control 31.2 ± 0.8 animals. The activity of glutathione peroxidase was less in aged control as compared to young control. Thus glutathione peroxidase activity decreased with age (Table 1). *Celastrus paniculatus* treatment increased the activity in aged treated animals. The rises in GPx activity in treated animals were greater than the age control as well as early age control.

Discussion

Ageing is characterised by gradual decline in biochemical processes, which determine structural and functional alterations at the cellular level[11]. The present study indicates that oxygen metabolism is good example of pleiotropic nature of ageing. While oxygen is essential for life it also produces free radicals, aldehydes and wide range of peroxides on the other hand[12-13]. There was a considerable decline in the activity of SOD in old animals. The decline in the activity of the enzyme was gradual with increasing age. These results are in agreement with earlier findings of Singh[14] and Vohra *et al.*[1]. All these authors have observed an age related decrease in the SOD activity of whole brain homogenates or different parts of the CNS in rat. Rao *et al* previously reported a 40% decrease in SOD activity in brain of the 26 months old male Fisher rat when compared with the 6 months old animal brain[15]. However, the magnitude of decrease between age control (20 months old) and young control (3 months old) varied upto 44.2% in cerebellum. This observation is in agreement with the report of Rao *et al.*[15], who have concluded that the activity of a particular enzyme present in several tissues may vary in a complex manner. Catalase is ubiquitous enzyme found in all known organisms. It catalyzes the breakdown of H_2O_2 to H_2O and molecular oxygen[1, 16]. An age related decline in catalase activity was observed in these studies. The results are in agreement with those of Rao *et al*[15] in rat brain and kidney. Cand and Verdeti[17] have reported a 20 % loss in catalase activity in 24 months old rat brain in comparison to that of the 4 months old young ones. In the present investigation activities of both these enzymes decreased with age. Michiels *et al*[18] reported a 30 % increase in survival when catalase was injected in combination with SOD, whereas the survival was only 21% and 4% for SOD and catalase respectively, when independently injected in human fibroblasts. The other major enzyme directly involved in antioxidant defence system is glutathione peroxidase (GPx). Although catalase and GPx are able to scavenge H_2O_2, GPx has a much higher affinity for H_2O_2 than catalase. Gul and co-workers suggest that H_2O_2 is prinicipally degraded by GPx in normal conditions[19,20]. In brain tissue the major protection against both

Table 1 Antioxidant enzymes activities in control and aged treated animals

Parameters	3 month old (Young control)	12 month old (Early age control)	20 month old (Age control)	20 month old (Aged, treated)
Super Oxide Dismutase •	14.7±0.5 (13.9–15.0)	11.3*±0.8 (10.4–12.4)	8.2*±0.3 (7.8–8.7)	8.7*±0.2 (8.5–8.9)
Catalase (μ mol/mg protein)	25.0±2.1 (23–28)	22.3±0.7 (21.3–23)	18.6*±0.9 (17.2–19.4)	22.0±0.8 (21–23)
Gluthione peroxidose (n mol of NADPH oxidized/ min/ mg protein)	47.2±0.8 (46.1–48.3)	39.6*±0.8 (38.3–40.3)	31.2*±0.8 (30.0–32.1)	40.6±0.3 (40.2–41.2)

Values are mean ± S.D. of three replicate. [$P < 0.01$ (t test)]

Figures in parenthesis show range

• *A unit of enzyme is defined as the amount of enzyme causing half of the maximum inhibition of nitobluetetrazolium. The values are given as the units of enzyme/mg protein.*

lipid peroxide and H_2O_2 is reported to be achieved by GPx[21]. GPx activity in our study revealed a steady reduction from young control to aged control. These observation are in agreement with the studies of Roy *et.al*[22] and Gul *et.al.*[19], who have reported a decrease in GPx activity with age in the cerebral hemisphere, cerebellum and the brain stem. Cand and Verdetti[17] reported decreased level of GPx with age in kidney and liver in rats, whereas Imre *et.al.*[23], reported a decrease in GPx activity with age in liver and kidney and many other tissues of mice. In an other study Benzi *et.al.*[24], found that GPx activity remained constant in the caudate putamen and the temporal cortex but decreased in the substantial nigra and the thalamus in rats. Hothorsall *et.al*[25] also found a marked decrease in GPx activity in the cerebral hemisphere in rats from 8 to 24 months old. Berr *et. al*[26] made similar observations on the blood in human subjects during ageing. Thus, a decrease in the activity of these antioxidant enzymes makes the central nervous system more susceptible to free radical attack. The differences in enzyme activity in different regions of the central nervous system indicate that the profile of any antioxidant enzyme markedly differs with age, in different regions of the CNS. This finding support the concept that different regions exhibit varying degree of vulnerability to the per-oxidative reactions as a function of age. *C. paniculatus* is an Ayurvedic drug which has shown promise in protecting the various age associated pathological changes in the brain. In the present investigation *C. paniculatus* was observed to induce various antioxidant enzymes. The enhanced activity of superoxide dismutase, catalase and glutathione peroxidase in the aged has proved to can be very effective in detoxifying the various types of oxygen free radicals and their products.

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Abbreviations

SOD - Super oxide dismutase, GPx - Glutathione peroxidise, CNS - Central nervous system, S.D. - Standard Deviation, NADPH - Nicotinamide adenine dinucleotide phosphate (reduced).

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