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RES ARTICLE

# Effect of *Celastrus paniculatus* on trace elements of cerebellum in ageing albino rats

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**ABSTRACT**

**Background**: In Indian traditional system of medicine *Celastrus paniculatus* extract has been used to improve intellect, memory and for the treatment of various mental disorders. **Purpose:** The present study was undertaken to evaluate the effectiveness of this medicinal plant on serum biochemistry. **Methods:** Ethanolic extract of seed of *Celastrus paniculatus* (2g/kg/body weight) was orally administrated for 16 days in 20 months old albino rats. The results were compared with 3 months, 12 months and 20 months old control rats. The concentration of trace elements was determined by atomic absorption spectrophotometer. **Results**: Significant variation was observed in the concentration of trace elements. In case of copper there was decrease in content in early aged (0.240 ± 0.004) control and age control (0.115 ± 0.004) rats whereas an increase in treated aged rats (0.124 ± 0.004) was observed. Non significant variation was observed in zinc content. Young control rats possessed 0.683 ± 0.004 (µg/ml) zinc contents in cerebellum. Age control animal showed the highest level of Zn 0.954 ± 0.002. Celastrus paniculatus treated rat show revealed the lowest level of zinc 0.457 ± 0.003 (µg/ml) in cerebellum. Young control rat had 0.066 ± 0 (µg/ml) manganese content which was significantly decreased in early age control (0.022 ± 0.0008) followed the significant increase in age control (0.087 ± 0.002). Treated rats possessed the decreased content than age control but higher than young and early age control. Non significant decrease in cobalt content was observed during ageing as in young control the highest cobalt content was 0.084 ± 0.0007 followed by decrease in early age control 0.83 ± 0 and age control 0.006 ± 0.0007 (µg/ml). Treated rats showed an increase in cobalt content up to 0.032 ± 0.0007. **Conclusion**: Results of the present study revealed that the determination of trace elements in blood and tissues has been widely used in the last two decades as a tool to understand their metabolic role in human and animals.

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## Introduction

The trace elements known to be essential for humans and unquestionably associated with deficiency symptoms include chromium, copper, iodine, iron, manganese, molybdenum, selenium and zinc.[1] Other trace elements such as arsenic, boron, cobalt, silicon, tin and vanadium have not been definitively linked to a specific deficiency. They all function within various human enzyme systems.[2] Taneja *et al* have reported increased frequency of atresia in rats on a zinc deficient feed.[3] Variations in zinc content are correlated to changes in distribution of specific carrier proteins and variation in albumin, dysregulation in the of activity of binding of selected hormones and interleukin. Low levels of zinc also enhance lipid peroxidation, thus, promoting ageing. Zinc deprivation also leads to an alteration in the structure and function of DNA as well as various regulatory proteins like RNAases and histones that play an important role in gene expression.[4] Elevated copper levels in females taking oral contraceptives is responsible for altering amine levels inducing physiological and behavioural alteration[5] and is associated with increased epinephrine and dopamine synthesis in brain and release of neurotransmitters.[6] A number of copper containing proteins and enzymes like cytochrome-C oxidase, superoxide dismutase, gluthathione peroxidase and transferase, tyrosine, dopamine, β-hydroxylase, amino oxidase, lysyl oxidase, ceruloplasmin and other enzymes of fatty acid metabolism[7] are known to function only in presence of copper. In addition butyryl CoA dehydrogenase, and fatty acid CoA dehydrogenase require copper traces for their activity.[7] There are nine trace elements believed to have major physiological role and of these iron, zinc and copper are among the most understood and worthy of concern.[9]

As our knowledge of trace elements grows, information about precise function and necessity will continue to emerge. Although, each of these elements has multiple physiological functions, in chronic excess they are dangerous to one's health. Because both deficiencies and overdoses are potentially dangerous, their intake must be monitored closely.[3]

## Methods

3 months old (young control), 12 months old (early age- control), 20 Months old (late age- control) and 20 months old (late age-treated) male Wister albino rats (weight 120-340g) were used in present study. Twelve-hour light and twelve-hour dark cycles along with 27 ± 2°C temperature conditions were maintained throughout the experiments. The animals were provided standard rat feed and water *ad libitum*. The study was approved by the Institutional animal ethic committee (Ref: IAEC/562/02/a/CPCSEA/24-11-2003)

### *Drug Preparation*

The seeds of *Celastrus paniculatus* were procured from an Ayurvedic medical practitioner at Kurukshetra as a single lot and sent to the Ayurvedic Department, Kurukshetra and Department of Botany, KUK for their verification and botanical identification. Powdered seeds were refluxed with ethyl alcohol (95%) in the ratio of 1:3 for 30 days with regular shaking 10-15 times per day. The extract was filtered using pressure vacuum pump, residual was refluxed again with ethyl alcohol (95%) and the filtered extract was collected. This process was repeated three times and the extract was pooled. The extract was then distilled under vacuum to remove all the traces of ethyl alcohol. Brown coloured oil was obtained in the trough. This was subsequently used for treatment of the experimental animals.

*Drug Schedule*

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

*Extraction*

For the extraction of trace elements, a minimum of 200mg of cerebellum tissue was digested in long necked round bottom flasks with triple acid (concentrated Nitric acid: 70% - perchloric acid: concentrated sulphuric acid, 10:3:1) in the ratio of 1:10 (w/v). The contents were heated till most of the triple acid mixture evaporated from the flask. The contents of each flask were then washed with 2ml of deionized water and were stored in plastic vials at 4°C for further analysis.

*Estimations*

The concentration of trace elements was determined by atomic absorption spectrophotometer installed at Sophisticated Analytical Instrumentation facility (SAIF), Panjab University, Chandigarh. The results obtained from various parameters were statistically analysed according to the mentioned statistical method.[10,11]

**Results**

Table 1 depicts the results obtained from trace element analyses in control and late age- treated animals. Significant variation was observed in the copper content in the cerebellum of all control and treated rats. In young control animals the copper content was 0.255 $\pm$ 0.004 ($\mu$g/ml). There was decrease in copper content in early age- control (0.240 $\pm$ 0.004) and late age-control (0.115 $\pm$ 0.004) rats. An increase in late age- treated rats (0.124 $\pm$ 0.004) was observed. Non significant variation was observed in zinc content. Young control rats possessed 0.683 $\pm$ 0.004 ($\mu$g/ml) zinc content in cerebellum. Early age- controls recorded decrease in zinc content 0.598 $\pm$ 0.002 ($\mu$g/ml). Late age- control animals showed the highest level of Zn 0.954 $\pm$ 0.002. *Celastrus paniculatus* treated rats showed the lowest level of zinc 0.457 $\pm$ 0.003 ($\mu$g/ml) in cerebellum. The highest iron 0.390 $\pm$ 0 content was observed in cerebellum of early age- controls. It was significant at the level of $P < 0.01$. In young control rats iron content was 0.364 $\pm$ 0.005 and significant decrease in late age-control 0.288 $\pm$ 0.005 rats was recorded. Treated rats showed increased iron levels 0.358 $\pm$ 0.07 ($\mu$g/ml). Manganese content significantly varied in control and treated rats. Young control rat had 0.066 $\pm$ 0 ($\mu$g/ml) manganese content. There was significant decrease in the manganese levels in early age- control (0.022 $\pm$ 0.0008) followed by significant increase in late age- control (0.087 $\pm$ 0.002). The late age- control animals showed the highest content of manganese. Treated rats showed decreased content than late age-control but higher than young and early age- control.

Non significant decrease in cobalt content was observed during ageing. In young control the highest cobalt content was 0.084 $\pm$ 0.0007 followed by decrease in early age- control 0.83 $\pm$ 0 and late age- control 0.006 $\pm$ 0.0007 ($\mu$g/ml). Treated rats showed an increase in cobalt content up to 0.032 $\pm$ 0.0007.

**Discussion**

Central nervous system controls and co-ordinates all body functions in an organism through its complex integrated circuits. In the present study, copper content decreased as the animal advanced in age. Decrease in copper concentration during ageing is in accordance with the findings of previous workers.[7,12] This decrease in copper content affects the activity of a number of enzymes such as cytochrome-C oxidase, glutathione peroxidase and transferase, tyrosine, dopamine-beta- hydroxylase, amino oxidase, lysyloxidase, ceruloplasmin, enzymes of the fatty acid metabolism, ascorbic acid oxidase, erythrocuprein, hepatocuprein butyl Co-A, dehydrogenase etc., which in turn alter the metabolic activity during development and ageing.[7,13] Although copper is not a part of haemoglobin molecule, yet it is involved in enhancing maturation of RBCs[14] and formation of haemogloblin and influences iron absorption. It is known to

**Table 1: Trace element analyses in control and late age- treated animals**

| Parameters | 3 month old (Young control) | 12 month old (Early age- control) | 20 month old (Late age- control) | 20 month old (Late Age- treated ) |
|---|---|---|---|---|
| Copper ($\mu$g/ml) | 0.255±0.004 (.250-.260) | 0.240*±.004 (.236-.247) | 0.115*±.004 (.110-.120) | 0.124*±.004 (.117-.128) |
| Zinc ($\mu$g/ml) | 0.683±0.004 (.677-.689) | 0.598±0.002 (.596-.601) | 0.954±.002 (.951-.957) | 0.457±.003 (.453-.462) |
| Iron ($\mu$g/ml) | 0.364±.005 (.357-.369) | 0.390*±0 (.390) | 0.288*±.005 (.282-.296) | 0.358±.07 (.351-.366) |
| Manganese ($\mu$g/ml) | 0.066±0 (.066) | 0.22*±.0008 (.021-.023) | 0.087*±.002 (.084-.089) | 0.081*±0008 (.080-.082) |
| Cobalt ($\mu$g/ml) | 0.084±.0007 (0.083-0.085) | 0.083±0 (.083) | 0.006±.0007 (.005-.007) | 0.032±.0007 (.031-.033) |

$P < 0.01$ (t test)

Values are mean $\pm$ S.D. of three replicate.

Figures in parenthesis show range

maintain the myelin sheath which surrounds nerve cells[15] Copper is a member of the respiratory chain and is involved in the formation of melanin pigments and is an important enzyme of catabolism that functions with the vitamin pantotheric acid at one end of the reaction chain and with cytochrome system at the other.[16] It is therefore, logical to deduce that variations observed in the copper titer are possibly due to some alterations in lipid metabolism (free radicals) and antioxidant defence enzymes inequity. This may induce a number of structural, physiological and biochemical alterations in the normal metabolic pathway possibly leading toward ageing.

High serum copper level in patients with diabetes mellitus may be attributed to hyperglycaemia that may stimulate glycosylation and release of more Cu ions that accelerates oxidative stress[17] and $Hb_{AIC}$ levels further contributes to the changes in the profile of other trace elements in blood. As a result of this, it contributes to the degree of higher oxidative stress in patients of diabetes mellitus.[18] Secondly, the fall in tissue Cu/Zn ratio adversely affects cytosolic super oxide dismutase resulting in alteration of antioxidant defence system.[19,20]

Induction of copper deficiency is an important enzymatic component of antioxidant defense system and increased lipid peroxidation may be a contributory factor in the pathophysiology of low Cu status.[21] In addition, various biochemical changes are believed to result from the decreased activity of Cu containing antioxidant enzymes, including super oxide dismutase, cytochrome-C oxidase, catalase and glutathione peroxidise.[22]

*C. paniculatus* treatment significantly ($P< 0.05$) enhances the activity of copper in late age- treated animals. Although this increase is slightly lower but positively correlated with young control. This increase was due to extra supplement in copper containing protein and enzymes to animals. Copper transporting ATPase of P type domains and N-terminal amino acid binding motifs regulate copper transport within the cell.[23]

The concentration of the zinc was lower in early age- control than young control rats. This decrease of zinc content in brain favour findings by Harrison *et al* and Danscher *et al*.[24,25] Cerebellar dysfunction has been associated with acute zinc loss.[26] Rats, zinc deficient in prenatal and early postnatal periods develop abnormal brain.[27] Decrease in zinc content affects the axonal transport, neuronal microtubule and tubulin synthesis and assembly.[28] Zinc deficiency during the critical period for brain growth permanently affects brain function, when this deficiency imposed is throughout the later part of pregnancy, brain size is decreased, there is a reduced total brain cell count and the cytoplasmic nuclear ratio is increased, implying an impairment of cell division in the brain.[29] The data of present study show that there was further increase in zinc content in late age- control and decrease the zinc concentration in treated animals.

The supplementation of Zn promotes food intake, linear growth and body weight increase.[30] High dose Zn supplementation in diabetes and normal individuals resulted in more hyperzincuria and increase in hemoglobin $A_{IC}$ in both diabetic and normal individuals.[31] Hypertension is a serious public health problem in the world. The higher the individual's blood pressure, the greater are the risks for developing heart disease, stroke, renal failure and peripheral vascular diseases.[32] Hypertension is an important risk factor for stroke and accelerates atherogenesis. There is strong evidence to support the idea that the rennin-angiotensin system (RAS) plays an important role in the pathogenesis of essential hypertension and its complications. Angiotensin converting enzyme (ACE) the most important component of rennin-angiotensin system, is usually associated with hypertension.[32] ACE is a well known Zn metallo-peptidase that converts angiotensin to the potent vasoconstrictor angiotensin II and that degrades bradykinin, a powerful vasodilator, both for the regulation of vascular tone and cardiac functions.[33] A direct increase in Zn levels in the plasma with increase in ACE activities may be the reason for elevated blood pressure or hypertension.[34]

We believe that the variation in Zn concentration in different age groups of rats is due to the alteration in their physiological requirement for Zn. The change is also associated with the fact that the physiological demand for Zn emanates as age advances.

The iron content in the cerebellum of control and treated animal showed an interesting trend. There was a significant ($P < 0.05$) increase in iron content in early age- control group. Increase in the iron concentration is due to the high metabolic activity in early age- control animals and support the finding of Pantopoulos *et al*.[9] It is well established that iron is a prerequisite for haemoglobin synthesis which is a vital pigment for the transport of oxygen.[35] Late age- control animal showed a decrease in iron content and *C. paniculatus* enhance the iron concentration in late age- treated rats. The decreased content in late age animals showed the impairment of physiological activities in late age animals. This iron deficiency causes a reduction in myoglobin, cytochrome-C, flavin containing enzymes, monoamine oxidase, x-1-glycerophosphate and other enzyme leading to impaired reduced bacteriocidal activity of neutrophills, impaired DNA synthesis, increased blood and urine catecholamines, and elevated level of thyroxine and reduced level of triido thyrionine[36] may in a pleiotropic manner induced the process of ageing. The drug treatment enhances the concentration of iron in late age- treated animals in accordance with the findings of Hebbrecht *et al*[37], which is due to the maintenance of iron homeostasis metabolism and physiological activity in late age animals.

No specific trend of variations in Manganese level was observed in the control and treated rats. There was a significant decrease in Mn concentration in early age- control. Mn deficiency effects cerebral motor function.[27] Huley *et al* demonstrated a relationship between seizure activity and Mn deficiency rats.[38] Tanaka has presented a preliminary report on low blood Mn levels in epileptic patients.[39]

Late age- control rats represent a significant increase in Mn level which decreased by drug administration. The enhanced levels are indicative of its utilization in enzyme activities like blood and bone phosphates, arginase required for the urea formation and as an activator of carboxylase, cholinesterase, muscle adenosine triphosphatase and other enzymes.[40] This increase may also be due to enhanced carbohydrate and protein metabolism involving Mn dependent intermediate reactions.[40]

The decreased level of Mn in drug treated animals are due to the poor Mn level in drug and the presence of high calcium and phosphate level.[41] Manganese is a cofactor in a number of enzymatic reactions, particularly those involved in phosphorylation, cholesterol and fatty acid synthesis.[4] It is established that during ageing cholesterol metabolism and fatty acid synthesis is severely affected.

Cobalt concentration decreased in the present investigation from young control to late age- control rats. The data revealed that there was a non significant lower level of cobalt in 20 months old rats. Decreased level of cobalt content during ageing support the earlier findings of Sharman and colleagues.[41] We strongly support the findings of Olivieri *et al* and KinCaid *et al*. They recorded increased Co concentration in Alzemier's patients compared with age matched control.[42,43] Investigation of present study is also in favor of cobalt as an inducer of oxidative stress/cell cytotoxicity and the resultant metabolic implications for neural cells. It is therefore good for general metabolism and cell that cobalt is reduced during ageing.

*C. paniculatus* treatment, however, increases the cobalt concentration in late age- treated rats. But this increase is negligible as compared to young control animal.

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**References**

1. Mertz W. The essential trace elements. Science 1981; 213 (4514): 1332–1338.
2. Carlisle EM. Silicon as an essential trace-element in animal nutrition, Ciba Foundsymp. 1986; 121: 123–139.
3. Taneja SK and Mahajan M. Zinc in obesity – A critical Review. JPAS 1999; 1: 211–216.
4. Nair N, Bedwal RS and Mathur RS. Biological trace elements: Their prophylactic, prognostic and etiological values. Cell Biol. News letter 1989; 11(1): 1–13.
5. Feller DJ and O'Dell BL. Dopamine and norepinephrine in discrete areas of copper deficient rat brain. J. Neurochem. 1980; 34: 1259–1261.
6. Prohaska JR, Bailey WR, Gross AM, *et al*. Effect of dietary copper deficiency on the distribution of dopamine and norepinephrine in mice and rats. J. Nutr. Biochem. 1990; 1: 149–154.
7. Sharma RK and Sharma M. Physiological perspectives of copper. Indian J. Exp. Biol. 1997; 35: 396–713.
8. Dangour AD, Sibson VL and Fletcher AE Micronutrient supplementation in later life: limited evidence for benefit. J. of Gerontology BS. 2004; 59a(7): 659–673.
9. Papanikolaou G and Pantopoulos K. Iron metabolism and toxicity. Toxicology and Applied Pharmacology 2005; 202: 199–211.
10. Panse VG and Sukhatme PV. In: Statistical methods for agricultural workers. 1985; ICAR Pub. New Delhi.
11. Zar JH. Biostatistical Analysis. 1984; Prentice-Hall Inc. Englehood Cliffs. N.J.
12. Pathak MM, Patel AV and Jana Kiraman K. Blood serum copper at different stages of pregnancy in Surti buffaloes. Ind. J. Anim. Sci. 1986; 56: 1202–1204.
13. Yur F, Bildik A, Belge F, *et al*. Serum, plasma and erythrocyte zinc levels in various animal species. YYU. Vet. Fak. Derg. 2002; 13(1-2): 82–83.
14. Johnson TW and Kramer TR. Effect of copper deficiency on erythrocyte membrane proteins of rats. J. Nutr. 1987; 117: 1085–1090.
15. Szerdahelyi P and Kasa P. Histochemical demonstration of copper in normal rat brain and spinal cord: Evidence of localization in glial cells. Histochem. 1986; 85: 341–347.
16. White A, Handler P, Smith EL, *et al*. Principles of Biochemistry. 1978; McGraw Hill Book Company, New York.
17. Mosaad A, Abou-Seif L and Abd-Allah Y. Evaluation of some biochemical changes in diabetic patients Clin. Acta. 2004; 346: 161–170.
18. Evliyaogly O, Kebapcilar L, Uzuncan N, *et al*. Correlation of serum $Cu^{+2}$, $Zn^{+2}$, $Mg^{+2}$ and $HbA_{Ic}$ in type 1 and type 2 diabetes mellitus. Turkish J. Endocrinol. Metab. 2004; 2: 75–79.
19. Nath N, Chari SN and Rathi AB. Super oxide dismutase in diabetic polymorpho nuclear leukocytes. Diabetes 1984; 33: 586–589.
20. Sandstead HH. Requirements and toxicity of essential trace elements, illustrated by zinc and copper. Am. J. Clin. Nutr. 1995; 61: 6215–6245.
21. Prohaska JR. Biochemical changes in copper deficiency J. Nutr. Biochem. 1990; 1: 453–461.
22. Rossi L, Lippe G, Marchesse E, *et al*. Decrease in CCO protein in heart mitochondria of copper deficient rats. Biomatal. 1998; 11: 207–212.
23. Neeru, Studies on intra-Follicular atretogenic factors in small ruminants. Ph.D. thesis, 2001 Kurukshetra University, Kurukshetra.
24. Harrison WW, Netsky G, and Brown MD. Trace elements in human brain: Copper, zinc, iron and magnesium. Clin. Chem. Acta. 1968; 21: 55–60.
25. Danscher G, Hall E, Fredens K, *et al*. Heavy metals in the amugdala of the rat : Zinc, lead and copper. Brain Res. 1975; 94: 167–172.
26. Henkin RI, Patten BM, Re PK, *et al*. A syndrome of acute zinc loss. Cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. Arch. Neurol. 1975; 32: 745–751.
27. Pfeiffer CC and LaMola BS. Zinc and Manganese in the Schizophrenias. Orthomolecular Psychiatry 1983; 12(3): 215–234.
28. Tamm LK, Crepeau H, and Edelstein SJ. Three dimensional reconstruction of tubulin in zinc induced Sheets. J. Mol. Biol. 1979; 130: 473.
29. Hurley LS, and Schrader RE. Congenital malformations of the nervous system in zinc-deficient rats in the International Review of Neurobiology, Pfeiffer CC (ed.) 1972 Academic Press, New York, 7.
30. McClain CJ, Kasarkis EJ Jr. and Allen JJ. Functional consequences of zinc deficiency. Prog. Food. Nutr. Sci. 1985; 9: 185–226.
31. Cunningham JJ, Fu A, Mearkte PL, *et al*. Hyperzincuria in invididuals with insulin dependent diabetes mellitus : Concurrent zinc status and the effect of high dose zinc supplementation. Metabol.1994; 43(12) : 1558–1562.
32. Edward JR, Giffaard RW and Alderman MD. The fifth report of the Joint National Committee on detection, evaluation and treatment of high blood pressure. Arch. Intern. Med. 1993; 153: 154–183.
33. Turner AJ and Hooper NM. The angiotensin converting enzyme gene family: genomics and pharmacology. Trends Pharmacol. Sci. 2002; 23(4): 177–183.
34. Ekmekci OB, Domma O and Tunckale A. Angiotensin converting enzyme and metals in untreated essential hypertension. Biol. Trace Elem. Res. 2003; 95: 203–210.
35. Sikka P. Role of minerls in reproduction – A Review, Ind. J. Diary Sci., 1992; 45: 159–167.
36. Pietrizik K, Prinz – Langenohl R and Thorand B. Micronutrients in pregnancy. Z. Geburtshilfe. Neonatol. 1997; 201(1): 21–24.
37. Hebbrecht G, Maenhaut W, and DeReuck J. Brain trace elements and aging. Nuclear Instruments and Methods in Physics Research 1999; 150(1-4): 208-213.
38. Huley LS, Wooley DE and Timiras PS. Threshold and Pattern of Electroshock Seizase in Ataxic Manganese Deficient Rats. Proc. Soc. Exp. Biol. Med. 1963; 106: 343–346.
39. Tanaka Y. Low Manganese level may trigger Epilepsy. JAMA 1977; 238: 1805.
40. Sangha GK, Sharma RK and Guraya SS. Distribution of trace elements in blood and ovary during the oestrous cycle and pregnancy in house rat. Ind. J. Anim. Sci. 1993; 63: 142–145.
41. Sherman WC. Manganese National Livestock and Meat Board. Food and Nutrition News 1965; 36: 8.
42. Olivieri G, Hers C, Savaskan E, *et al*. Melatonin protects SHSY5Y neuroblastoma cells from cobalt induced oxidative stress, neurotoxicity and increased β-amyloid secretion. J. of Pineal Res. 2001; 31(4): 320–325.
43. KinCaid RL, Lefebvre LE, Cronrath JD, *et al*. Effect of dietary Cobalt supplementation on Cobalt metabolism and performance of dairy cattle. J. Diary Sc. 2003; 86(4): 1405–14.