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Original Research

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Effect of aspartame in spinal cord and motor behavior in Wistar albino rats

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ABSTRACT

Introduction: More than 90 countries have given the artificial sweetener aspartame the green light to be used in thousands of food and beverage products. Two hundred times sweeter than sugar, aspartame allows food manufacturers to produce sweet foods they can market as "low calorie," "diet," or sugar-free," appealing to hundreds of millions of consumers looking to cut sugar from their diets. Concern relating to the possible adverse effect has been raised due to aspartames metabolic components. Aspartame is rapidly and completely metabolized in humans and experimental animals to aspartic acid (40%), phenylalanine (50%) and methanol (10%). Methanol, a toxic metabolite is primarily metabolized by oxidation to formaldehyde and then to formate these processes are accompanied by the formation of superoxide anion and hydrogen peroxide. **Lacuna and Method:** This study focus is to understand whether the oral administration of aspartame (40mg/kg.bw) for 90 days, has any effect on membrane bound ATPases, antioxidant status(both enzymatic and non-enzymatic) in spinal cord and motor behavior of Wistar albino rats. To mimic human methanol metabolism, folate deficient rats were used. **Result:** After 90 days of aspartame administration, showed a significant alteration in membrane bound ATPases, decrease in both enzymatic and non-enzymatic antioxidant level while there was no significant change was observed in motor behavior. **Conclusion:** This study concludes that oral administration of aspartame (40mg/kg.bw) for longer duration may cause oxidative stress in spinal cord, which didn't have any consequence on motor behavior, but which may be the root of other neuronal complication because oxidative stress in spinal cord can't be ignored.

KEY WORDS: Aspartame, ATPase's, oxidative stress, motor behavior, spinal cord

INTRODUCTION

Many non-nutritive sweeteners have been used in foods and beverages to allow people to enjoy the sweet taste without consuming sugar-associated calories. One of these sweeteners is aspartame (ASP). This sweetener and its metabolic breakdown products (phenylalanine, aspartic acid, and methanol) have been a matter of extensive investigation for more than 20 years including experimental animal studies. Aspartic acid and phenylalanine, because it easily crosses the blood/brain barrier, is neurotoxic when unaccompanied by the other amino acids in proteins. 10% of ASP consists of methanol. Methanol is a deadly poison. It is found in wood alcohol. Methanol is gradually released in the small intestine when the methyl group of ASP encounters the enzyme chymotrypsin. This affects the dopamine system of the brain causing addiction. The daily intake of ASP is estimated to be 2.5-5 mg/kg body weight [1]. ASP was approved by the U.S. Food and Drug Administration in 1981 for dry food [2] in 1983 for soft drinks [3], and in 1996 for all foods [4]. In 1994, it was approved for use throughout the European Union [5]. ASP is metabolized in the gastrointestinal tract by esterases and peptidases into three components: The amino acids phenylalanine and aspartic acid, and methanol [6]. ASP can be also absorbed into the mucosal cells prior to hydrolysis followed by metabolized within the cell to its three components, which then enter circulation [7].

The coordination of motor output depends critically on sensory feedback information provided by proprioceptive sensory neurons. The selectivity of proprioceptive afferent–motor neuron connectivity has its basis in the formation of distinct afferent termination zones in the spinal cord, as well as in recognition of specific motor neuron targets. Hence, we have been a focus on the spinal cord involved in the control of motor movement. Spinal cord is highly vulnerable to oxidative damage. Central nervous system has a rich source of Lipids and may be the predominant target of free radical mediated lipid peroxidation (LPO). Central nervous system is particularly susceptible to the toxic effect of oxygen free radical due to low level of antioxidant enzyme and glutathione (GSH), readily oxidizable substances, such as polyunsaturated fatty acid (PUFA) and catecholamine and high rate of oxidative metabolic activity [8]. LPO, a marker for oxidative damage, is associated with a progressive loss in membrane fluidity, reduction in membrane potential, increase in membrane permeability to ions, alteration of membrane bound ATPases enzymes, which finally leads to cellular damage [9]. Elevated levels of LPO are responsible for the decreased physiological performance and increased susceptibility to disease and death [10]. Despite numerous toxicological studies of ASP; its effect on the spinal cord and its locomotors behavior have be given little attention. Therefore, this study was designed to investigate cellular

damage, by altering membrane ATPases, LPO, and anti-oxidant enzyme in spinal cord of Wistar albino male animals on exposure of ASP (40 mg/kg body weight).

METHODS

Animal Model

Animal experiments were carried out after getting clearance from the Institutional Animal Ethical Committee (IAEC No: 02/03/11) and the Committee for the Purpose of Control and Supervision of Experiments on Animals. The experimental animals were healthy, inbreed adult male Wistar albino rats, weighing approximately 200-220 g (12 week of age). The animals were maintained under standard laboratory conditions and were allowed to have food and water *ad libitum* (standard rat feed pellets supplied by M/s. Hindustan Lever Ltd., India) for control animals and but for folate deficient (FD) were given special FD diet for 37 days [11] and methotrexate (MTX) (0.1 mg/100 kg body weight) i.p. every other day for 2 week [12] before euthanasia. Animals of ASP treated groups were daily administered ASP (40 mg/kg body weight) [5] dissolved in normal saline orally (by means of gavage needle) for 15-day 30-day and 90-day. All the rats were housed under the condition of controlled temperature (26 ± 2°C) with 12 h light and 12 h dark exposure.

Experimental Design

Group I was the control animals (control group), which were administered normal saline orally (by means of lavage needle) thought out the experimental protocol. Group III was control animals treated with ASP (40 mg/kg body weight) for 90 days (Cont+ ASP). Since human beings have very low hepatic folate content [13]. In methanol metabolism conversion of formate to carbon dioxide is folate dependent. Hence in the deficiency of folic acid, methanol metabolism could take the alternate pathway (microsomal pathway) [14]. To simulate this, rats were made FD by feeding them on a special dietary regime for 37 days and after that MTX in sterile saline was administered by every other day for 2 week [12] before euthanasia. MTX folate deficiency was confirmed by estimating the urinary excretion of formaminoglutamic acid (FIGLU) [15] prior to the experiment. Rats on a FD diet excreted an average of 70 mg FIGLU/kg body weight/day (range 25-125), while animals on the control diet excreted an average of 0.29 mg/kg body weight/day (range 0.15-0.55). These FD animals showed a significant increase in FIGLU excretion when compared to the control animals ($P \leq 0.05$). The folate deficient animals were further divided into 2 groups. Group II was FD diet fed control, (FD group) Group IV was FD animals treated with ASP (40 mg/kg body weight) for 90 days (FD + ASP).

Group I: Control animals.
Group II: FD control animal.
Group III: Control animals treated with ASP (40 mg/kg body weight) orally for 90 days.
Group IV: FD animals treated with ASP (40 mg/kg body weight) orally for 90 days.

Sample Collection

All the animals were sacrificed under deep anesthesia using pentothal sodium (40 mg/kg body weight). Isolation of spinal cord was performed between 8 and 10 a.m. to avoid circadian rhythm induced changes and were homogenized by using Teflon glass homogenizers. 10% homogenate of was prepared in phosphate buffer (0.1 M, pH 7.0) and centrifuged at 3000 g at 4°C for 15 min to remove cell debris and the clear supernatant was used for further biochemical assays.

Biochemical Determinations

The activity of (ATPase) Na^+/K^+ ATPase (EC 3.6.1.3) was estimated by the method of Bonting, [16]. Ca^{2+} ATPase (EC 3.6.1.3) by the method of Hjertén and Pan [17] and Mg^{2+} ATPase (EC 3.6.1.3) by the method of Ohnishi *et al.* [18], in which the liberated phosphate was estimated according to the method of Fiske and Subbarow [19]. Protein was estimated as per the method described by Lowry *et al.* [20], nitric oxide (NO) levels were measured as total nitrite + nitrate levels with the use of the Griess reagent by the method of Green *et al.* [21]. LPO was determined as described by Ohkawa *et al.* [22], superoxide dismutase (SOD) (EC.1.15.1.1) according to (Marklund and Marklund [23] and catalase [CAT]) (EC. 1.11.1.6) according to the method of Sinha [24]. The activity of glutathione peroxidase (GPx) (EC.1.11.1.9) was estimated by the methods of Rotruck *et al.* [25]. Reduced GSH was estimated by the method of Moron [26]. The vitamin-C (ascorbic acid) was determined according to the method of Omaye *et al.* [27].

Evaluation of Motor Coordination

The assessment of motor coordination was performed on day 15-day, 30-day, 45-day, 60-day, 75-day and 90-day using Rota rod was according to the descriptions of Dunham and Miya [28]. The beam walk performance task as described by Hicks and D'Amato [29] and Grid walking test by Behrmann *et al.* and Metz *et al.* [30,31].

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). All data were analyzed using the SPSS for windows statistical package (version 20.0, SPSS Institute Inc., Cary, North Carolina, USA). Statistical significance between the different groups was determined by one-way analysis of variance (ANOVA). When the groups showed a significant difference then Tukey's multiple comparison tests was followed, and the significance level was fixed at $P \leq 0.05$.

RESULTS

Effect of ASP on Membrane Bound Enzymes

The data are summarized in [Table 1] with mean ± SD. The membrane bound enzymes (Na^+K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase) in spinal cord of FD animals was similar to control animals. However, the control animals as well as FD diet fed animals treated with ASP for 90-day, the entire

membrane bound enzymes (Na^+K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase) of spinal cord was decreased when compared with controls and FD diet fed animals.

Effect of ASP on LPO and NO Level

The results of lipid per oxidation in spinal cord are summarized in Table 2 as mean ± SD. The LPO and NO level of FD animals was similar to the control animals. However, the control animals as well as FD diet fed animals treated with ASP for 90-day, the LPO level and NO level of spinal cord was increased when compared with controls and FD diet fed animals. This clearly indicates the generation of free radicals by ASP.

Effect of ASP on Enzymatic and Non-enzymatic Antioxidant Level

The results of enzymatic and non-enzymatic antioxidant level in spinal cord are summarized in Table 2 with mean ± SD. All enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and vitamin C) antioxidants level didn't get altered in the FD diet fed animal when compared with control animal. But, the control animals as well as FD diet fed animals treated with ASP for 90-day, enzymatic (SOD, CAT and GPx) and non-enzymatic (GSH and vitamin C) antioxidants level were significantly decreased when compared to control, and FD diet fed animal.

Effect of ASP on Motor Co-ordination Test

Rota rod, narrow beam and grid runway: The result obtained from the experiment is presented as bar diagram in Figures 1-3. With mean ± SD. The retention time on revolving rod was similar in control animals as well as in the FD diet fed animals. Even in ASP treated animals for 90-day also did not show any significant change when compared with control as well as FD diet fed animal. The result shows that ASP did not have an effect on motor behavior.

The time taken to cross narrow beam and grid runway was similar in control animals as well as in the FD diet fed animals. Even in ASP treated animals for 90-day also did not show any significant change when compared with control as well as FD diet fed animal. The result shows that ASP didn't have an effect on motor behavior.

DISCUSSION

In this study, the FD diet fed animals were used to mimic the human methanol metabolism. However, the FD diet fed animals did not showed any significant changes in the parameters studied and remained similar to controls. Oxidative stress can cause the primary injury to the spinal cord [32]. Antioxidant defense mechanisms are important to prevent the potential harmful effects of oxidative stress [33]. Cell membranes are rich sources of PUFAs. The oxidative destruction of PUFAs is known as LPO, which causes tissue injury. This alteration after ASP administration may be attributed to its metabolite methanol. Methanol is primarily metabolized by oxidation to formaldehyde and then to formate, these processes are accompanied by the formation of superoxide anion and hydrogen peroxide [34], which is also again substantiate by the elevated NO level in this study. NO is thought to react with superoxide anion to gain a radical property, which is a potent source of oxidative injury [35]. Membrane ATPase's, which control ions gradient across organelle and plasma membrane appear to be particularly susceptible to oxidative change. In this study in the ASP treated groups, there was decreases in membrane bound Na^+/K^+; Mg^{2+}; and Ca^{2+} ATPase's activities. The modified enzymatic and non-enzymatic free radical scavenging system with an elevated LPO and NO level after ASP administration clearly indicated the generation of free radicals in the present study. SOD form the primary defense against reactive oxygen metabolites its plays a key role in detoxifying superoxide anions, which otherwise damages the cell membranes and macromolecules. CAT has the capability to detoxify H_2O_2 radicals. Release of H_2O_2 promotes the formation of numerous other oxidant species that greatly contributes for oxidative stress. The over expression of CAT in neuronal cells provides protection against H_2O_2-induced toxicity that subsequently leads to the activation of endonuendonuclease digestion of DNA into oligosomes [36]. GPx catalyzes the reaction of hydroperoxides with GSH to form GSH disulfide. GPx, which uses GSH as a proton donor,

Table 1: Effect of aspartame on membrane ATPases of spinal cord

Parameters	Cont	FD	Cont+ASP	FD+ASP
Na^+k^+ ATPase#	2.56±0.30	2.39±0.25	0.99±0.13*a,*b	0.93±0.14*a,*b
Ca^{2+}ATPase#	1.85±0.36	1.72±0.30	0.65±0.37*a,*b	0.51±0.34*a,*b
Mg^{2+}ATPase#	1.18±0.41	1.16±0.09	0.06±0.034*a,*b	0.09±0.10*a,*b

Each value represents mean±SD. Significance at *$P \leq 0.05$, #μ moles of inorganic phosphate lib/min/mg protein, *[a]Compared with control, *[b]Compared with FD group, Cont: Control, FD: Folate deficient, ASP: Aspartame, SD: Standard deviation

Table 2: Effect of aspartame on antioxidant parameter of spinal cord

Parameters	Cont	FD	Cont+ASP	FD+ASP
LPO (MDA[nmol/mg protein])	0.51±0.08	0.55±0.07	2.98±0.38*a,*b	2.89±0.34*a,*b
Nitric oxide (μmoles of nitrite/mg protein)	5.55±0.57	5.79±0.45	13.75±0.52*a,*b	13.18±0.63*a,*b
SOD (units/mg protein)	1.80±0.22	1.81±0.25	1.23±0.16*a,*b	1.12±0.15*a,*b
Catalase (μ moles of H_2O_2 consumed/mg protein)	3.36±0.21	3.31±0.24	2.54±0.24*a,*b	2.48±0.17*a,*b
GPX (μg of GSH consumed/mg protein)	3.85±0.29	3.95±0.26	2.18±0.22*a,*b	2.08±0.17*a,*b
GSH (μg/mg protein)	0.068±0.015	0.062±0.012	0.0056±0.001*a,*b	0.0058±0.003*a,*b
Vitamin C (μg/mg protein)	0.590±0.057	0.586±0.043	0.0863±0.04*a,*b	0.102±0.123*a,*b

Each value represents mean±SD. Significance at *$P \leq 0.05$, *[a]Compared with control, *[b]Compared with FD group, Cont: Control, FD: Folate deficient, ASP: Aspartame, SD: Standard deviation, LPO: Lipid peroxidation, SOD: Superoxide dismutase, GPX: Glutathione peroxidase, GSH: Glutathione

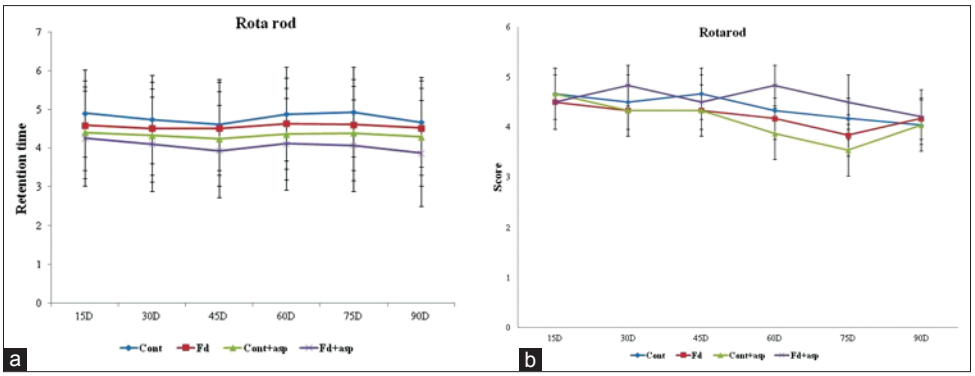

Figure 1: (a and b) Each value represents mean ± standard deviation. Significance at **P*<0.05. Cont: Control, FD: Folate deficient, ASP: Aspartame, D: Number of days. Score 5: Normal (balanced with steady position), 4: Grasp side of the beam, 3: One limb falls down from the beam or legs on beam, 2: Attempt to balance on the beam but falls off (>40 s), 1: Attempt to balance on the beam but falls off (>20 s), 0: Falls off no attempt to balance or hang on beam

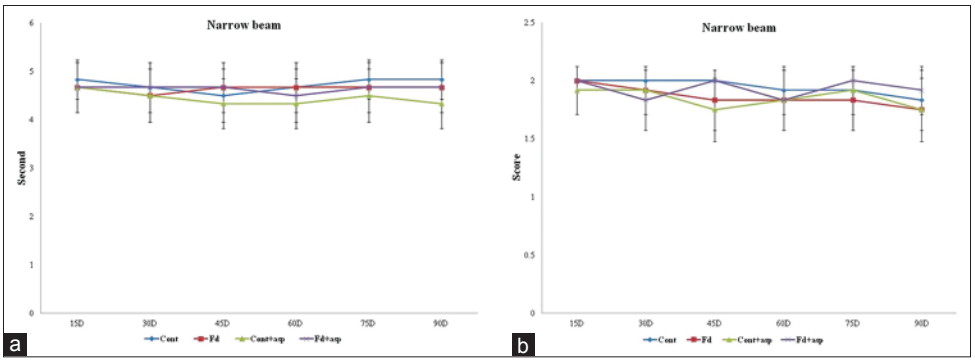

Figure 2: (a and b) Each value represents mean ± standard deviation. Significance at **P*<0.05. Time to cross narrow beam is expressed in second. Cont: Control, FD: Folate deficient, ASP: Aspartame, D: Number of days. Score: Nature of crossing the beam with both limbs, 2.0: Without any slip, 1.5: With slip between 1 and 3, 1.0: With slip between 3 and 6, 0.5: With slip between 7 and 10, 0.0: With slip more than 10

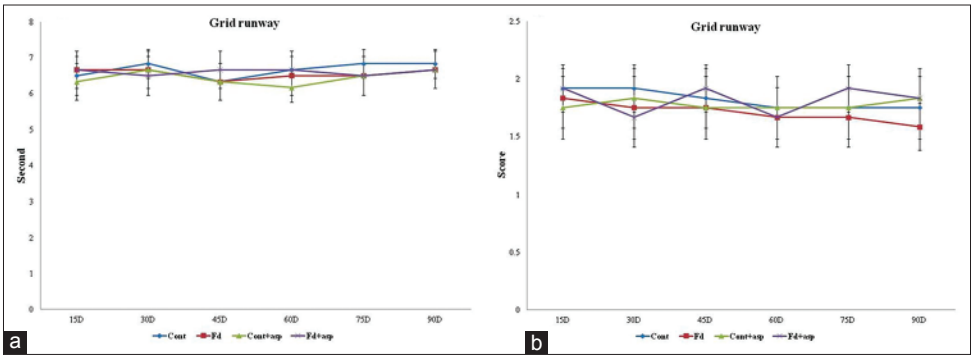

Figure 3: (a and b) Each value represents mean ± standard deviation. Significance at **P*<0.05, Time to cross grid runway is expressed in second. Cont: Control, FD: Folate deficient, ASP: Aspartame, D: Number of days. Score: Nature of crossing the grid runway with both limbs, 2.0: Without any slip, 1.5: With slip between 1 and 3, 1.0: With slip between 3 and 6, 0.5: With slip between 7 and 10, 0.0: With slip more than 10

converts H_2O_2 to water and molecular oxygen; in this process GSH is oxidized to GSSG.

Apart from enzymic antioxidants, various non-enzymic antioxidants also play an important role in preventing cells from oxidative threats. In the present study, the levels of non enzymic antioxidants like GSH and vitamin C were significantly decreased in ASP treated rats. The decrease in GSH levels in spinal cord [37] represents increased Susceptibility to oxidative stress. GSH, a tripeptide composed of l-glutamate, l-cysteine and l-glycine may act as a redox modulator of ionic receptors that serve as a neuroprotectant in glutamatergic excitotoxicity, and as neurotransmitters. The protective capacity of GSH sulfhydryl cysteine moiety, which can bind to electrophilic sites on xenobiotics and endogenous toxins [38] vitamin C (ascorbate) belonging to the water-soluble class of vitamins, readily donates electrons to break the chain reaction of LPO. Furthermore, ascorbic acid regulates the activity of some neurons

within the brain and spinal cord. Some of these functions include neurotransmitter membrane receptor synthesis, and neurotransmitter dynamics [39] ASP treated animals for 90-day did not show any significant change in motor behavior when compared with control as well as FD diet fed animal. This shows that Exposure to ASP has no effect on motor behavior.

CONCLUSION

The results of this study thus indicate that long-term intake of oral administration of ASP (40 mg/kg body weight) lead to labialization of the cell plasma membrane, Such as disruption of the ordered lipid bilayer of the plasma membrane, and induced oxidative stress. This shows that long-term intake of ASP cause oxidative stress on the spinal cord and which did not have any effect on motor behavior. These alterations in spinal cord may be due to the production of the high number of free radical by methanol metabolite of ASP, but which may cause other complication due to oxidative stress in spinal cord.

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REFERENCES

1. Butchko HH, Stargel WW, Comer CP, Mayhew DA, Benninger C, Blackburn GL, *et al*. Intake of aspartame vs. the acceptable daily intake. Regul Toxicol Pharmacol 2002;35:S13-6.
2. Food and Drug Administration (FDA). Aspartame: Commissioner's final decision. Fed Regist 1981;46:38285-308.
3. Food and Drug Administration (FDA). Food additives permitted for direct addition to food for human consumption: Aspartame. Fed Regist 1983;48:31376-82.
4. Food and Drug Administration (FDA). Food additives permitted for direct addition to food for human consumption: Aspartame. Fed Regist 1996;61:33654-6.
5. European Food Safety Authority (EFSA). Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to a new long-term carcinogenicity study on aspartame. Eur Food Saf Authority J 2006;356:1-44.
6. Ranney RE, Oppermann JA, Muldoon E, McMahon FG. Comparative metabolism of aspartame in experimental animals and humans. J Toxicol Environ Health 1976;2:441-51.
7. Mattews DM. Absorption of peptides, amino acids, and their methylated derivatives. In: Stegink LD, Filer LJ Jr, editors. Aspartame Physiology and Biochemistry. New York: Dekker; 1984. p. 29-46.
8. Evans PH. Free radicals in brain metabolism and pathology. Br Med Bull 1993;49:577-87.
9. Yu BP, Yang R. Critical evaluation of the free radical theory of aging. A proposal for the oxidative stress hypothesis. Ann N Y Acad Sci 1996;786:1-11.
10. Spiteller G. Lipid peroxidation in aging and age-dependent diseases. Exp Gerontol 2001;36:1425-57.
11. Walzem RL, Clifford AJ. Folate deficiency in rats fed diets containing free amino acids or intact proteins. J Nutr 1988;118:1089-96.
12. Ming H, Wang SY, Meadows CA, Thenen SW. Methotrexate effects on folate status and the deoxyuridine suppression test in rats. Nutr Res 1989;9:431-44.
13. Makar AB, Tephly TR. Methanol poisoning in the folate-deficient rat. Nature 1976;261:715-6.
14. Tephly TR. The toxicity of methanol. Life Sci 1991;48:1031-41.
15. Rabinowitz JC, Pricer WE. Formiminotetrahydrofolic acid and methenyltetrahydrofolic acid as intermediates in the formation of N10-formyltetrahydrofolic acid. J Am Chem Soc 1956;78:5702-4.
16. Bonting SL. Sodium potassium activated adenosine triphosphatase and cation transport. In: Bitler EE, editor. Membrane and Ion Transport. Vol. 1. London: Interscience Wiley; 1970.p. 257-63.
17. Hjertén S, Pan H. Purification and characterization of two forms of a low-affinity Ca^{2+}-ATPase from erythrocyte membranes. Biochim Biophys Acta 1983;728:281-8.
18. Ohnishi T, Suzuki T, Suzuki Y, Ozawa K. A comparative study of plasma membrane Mg2+ -ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta 1982;684:67-74.
19. Fiske CH, Subbarow Y. The colorimetric determination of phosphorous. J Biol Chem 1925;663:75-400.
20. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
21. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
22. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
23. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem 1974;47:469-74.
24. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
25. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
26. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
27. Omaye ST, Turnbull JD, Sauberlich HE. Selected methods for the determination of ascorbic acid in animal cells, tissues, and fluids. Methods Enzymol 1979;62:3-11.
28. Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficit in rats and mice. J Am Pharm Assoc Am Pharm Assoc (Baltim) 1957;46:208-9.
29. Hicks SP, D'Amato CJ. Motor-sensory cortex-corticospinal system and developing locomotion and placing in rats. Am J Anat 1975;143:1-42.
30. Behrmann DL, Bresnahan JC, Beattie MS, Shah BR. Spinal cord injury produced by consistent mechanical displacement of the cord in rats: Behavioral and histologic analysis. J Neurotrauma 1992;9:197-217.
31. Metz GA, Merkler D, Dietz V, Schwab ME, Fouad K. Efficient testing of motor function in spinal cord injured rats. Brain Res 2000;883:165-77.
32. Hall ED, Springer JE. Neuroprotection and acute spinal cord injury: A reappraisal. NeuroRx 2004;1:80-100.
33. Yücel N, Cayli SR, Ates O, Karadag N, Firat S, Turköz Y. Evaluation of the neuroprotective effects of citicoline after experimental spinal cord injury: Improved behavioral and neuroanatomical recovery. Neurochem Res 2006;31:767-75.
34. Parthasarathy NJ, Kumar RS, Manikandan S, Devi RS. Methanol-induced oxidative stress in rat lymphoid organs. J Occup Health 2006;48:20-7.
35. Jaeschke H. Role of reactive oxygen species in hepatic ischemia-reperfusion injury and preconditioning. J Invest Surg 2003;16:127-40.
36. Mann H, McCoy MT, Subramaniam J, Van Remmen H, Cadet JL. Overexpression of superoxide dismutase and catalase in immortalized neural cells: Toxic effects of hydrogen peroxide. Brain Res 1997;770:163-8.
37. Kamencic H, Griebel RW, Lyon AW, Paterson PG, Juurlink BH. Promoting glutathione synthesis after spinal cord trauma decreases secondary damage and promotes retention of function. FASEB J 2001;15:243-50.
38. Mitchell JR, Jollow DJ, Potter WZ, Gillette JR, Brodie BB. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J Pharmacol Exp Ther 1973;187:211-7.
39. Li X, Cobb CE, Hill KE, Burk RF, May JM. Mitochondrial uptake and recycling of ascorbic acid. Arch Biochem Biophys 2001;387:143-53.

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