Phenolic antioxidants attenuate hippocampal neuronal cell damage against kainic acid induced excitotoxicity

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Increasing evidence supports the role of excitotoxicity in neuronal cell injury. Thus, it is extremely important to explore methods to retard or reverse excitotoxic neuronal injury. In this regard, certain dietary compounds are begining to receive increased attention, in particular those involving phytochemicals found in medicinal plants in alleviating neuronal injury. In the present study, we examined whether medicinal plant extracts protect neurons against excitotoxic lesions induced by kainic acid (KA) in female Swiss albino mice. Mice were anesthetized with ketamine and xylazine (200 mg and 2 mg/kg body wt. respectively) and KA (0·25 μg in a volume of 0·5 μl) was administered to mice by intra hippocampal injections. The results showed an impairment of the hippocampus region of brain after KA injection. The lipid peroxidation and protein carbonyl content were significantly ($P < 0·05$) increased in comparison to controls. Glutathione peroxidase (GPx) activity (EC 1·11·1·9) and reduced glutathione (GSH) content declined after appearance of excitotoxic lesions. As GPx and GSH represent a major pathway in the cell for metabolizing hydrogen peroxide (H_2O_2), their depletion would be expected to allow H_2O_2 to accumulate to toxic levels. Dried ethanolic plant extracts of *Withania somnifera* (WS), *Convolvulus pleuricauas* (CP) and *Aloe vera* (AV) dissolved in distilled water were tested for their total antioxidant activity. The diet was prepared in terms of total antioxidant activity of plant extracts. The iron (Fe^{3+}) reducing activity of plant extracts was also tested and it was found that WS and AV were potent reductants of Fe^{3+} at pH 5·5. CP had lower Fe^{3+} reducing activity in comparison to WS and AV. Plant extracts given singly and in combination 3 weeks prior to KA injections resulted in a decrease in neurotoxicity. Measures of lipid peroxidation and protein carbonyl declined. GPx activity and GSH content were elevated in hippocampus supplemented with WS and combination of WS + CP + AV. However, when CP and AV were given alone, the changes in the GPx activity and GSH content were not significant. Although the major factors involved in these properties of phytochemicals remain to be specified, the finding of this study has suggested that phytochemicals present in plant extracts mitigate the effects of excitotoxicity and oxidative damage in hippocampus and this might be accomplished by their antioxidative properties.

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1. Introduction

Excitotoxicity is a contributing factor to the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (Law *et al* 2001) and Parkinson's disease (Beal 1995; Hantraye *et al* 1996). Although each disease has distinctive morphological and biochemical characteristics, the pathology of each is consistent with oxidative

Keywords. Excitotoxicity; hippocampus; neurodegeneration; oxidative stress; phytochemicals; reactive oxygen species

Abbreviations used: AV, *Aloe vera*; CP, *Convolvulus pleuricauas*; DNPH, 2,4-dinitrophenyl hydrazine; FRAP, ferric reducing/antioxidant power; GPx, glutathione peroxidase; GSH, glutathione; KA, kainic acid; MDA, malonaldehyde equivalent; $O_2^{\cdot-}$, superoxide radical; WS, *Withania somnifera*.

damage. It is thought that there are many neurochemical modulators of nervous system damage. In epilepsy, excessive glutamate-mediated neurotransmission, impaired voltage-sensitive sodium- and calcium-channel functioning as well as impaired GABA-mediated inhibition and alterations in acid-base balance, may trigger a cascade of events leading to neuronal damage and cell death (Vajda 2002). Studies directly linked glutamate-induced increases in cellular calcium to mitochondrial free radical generation. Free radical generation has been demonstrated using several redox-sensitive dyes; for example dichlorofluoroscein and dihydroethidine (Bindokas *et al* 1996; Reynolds and Hastings 1995). The role of superoxide anion radical ($O_2^{\cdot-}$) in excitotoxic injury was studied by examining effects on aconitase, a tricarboxylic acid cycle enzyme that contains iron-sulphur clusters inactivated by $O_2^{\cdot-}$ (Patel *et al* 1996). Treatment of rat cortical cultures with (NMDA), kainic acid (KA) or an intracellular $O_2^{\cdot-}$ generator produced a selective and reversible inactivation of aconitase that closely correlated with subsequent cell death (Beal 1997).

Under normal conditions, damage by oxygen radicals is kept in check by an efficient array of antioxidant systems. However, during pathological conditions, the oxidant vs antioxidant balance is altered, either primarily or secondarily. It has been demonstrated that certain phenolic antioxidants attenuate neuronal cell death induced by oxidative stress (Schroeter *et al* 2000; Youdim and Joseph 2001). The antioxidant properties of green tea extract (Hong *et al* 2000), blueberry, spinach or strawberry (Joseph *et al* 1999), *Allium sativum* (Pedraza-Chaverri *et al* 1998) and *Glycyrrhiza glabra* (Vaya *et al* 1997) have already proven beneficial in reducing oxidative tissue injury. Such studies yield valuable nutritional information and validate those studies which have already reported positive effects.

The present study has been designed to investigate the role of polyphenolic dietary supplementation extracted from *Withania somnifera* (WS) *Convolvulus pleuricauas* (CP) and *Aloe vera* (AV) on the protection of neuronal cells from injury induced by excitotoxicity in mice.

2. Materials and methods

2.1 *Reagents*

Trizma base, trizma HCl, 5-5-dithiobis-[2-nitrobenzoic acid), KA, bovine serum albumin (BSA) and glutathione reductase were obtained from Sigma, St. Louis, MO, USA. Sodium dodecyl sulphate (SDS), acetic acid, n-butanol, pyridine and EDTA were obtained from Glaxo Chemicals, India. Reduced glutathione (GSH), thiobarbituric acid and trichloroacetic acid (TCA) were obtained from Loba Chemi, India. 2,4-dinitrophenyl hydrazine (DNPH), guinidine hydrochloride, NADPH, H_2O_2 and ethyl acetate were obtained from Hi-Media Laboratories, India. FRAP reagent [300 mM acetate buffer (pH 3·6) + 16 ml glacial acetic acid (made up to 1 liter with distilled water); 10 mM 2,4,6-tripyridal-S-triazine (TPTZ, Fluca, Switzerland) in 40 mM HCl; and 20 mM $FeCl_3$. $6H_2O$ in the ratio of 10 : 1 : 1. All other chemicals used were of analytical grade.

2.2 *Animals*

Female Swiss albino mice (*Mus musculus albinus*) weighing 25–30 g were housed in an environmentally regulated room on a 12 h light : 12 h dark cycle with 25 ± 1ºC and had free access to food and water. The use of animals in experimental protocol was approved by the research committee of the Vikram University, Ujjain, in accordance with the international guidelines for the care and use of laboratory animals.

2.3 *Experimental design*

A total of 72 mice were randomly divided into nine groups with 8 mice in each group. Mice in group I were controls. Groups II, III and IV were treated with alcoholic extracts (in distilled water) of WS, CP and AV respectively. Group V received an intra-hippocampal injection of KA. Group VI received the intra-hippocampal injection of KA + oral administration of WS. Group VII received the intra-hippocampal injection of KA + oral administration of CP. Group VIII received the intra-hippocampal injection of KA + oral administration of AV. Group IX received the intra-hippocampal injection of KA + oral administration of WS + CP + AV combination.

2.4 *Plant materials*

The powdered roots of WS, powdered whole plant of CP and fleshy inner part of AV leaves were extracted in acetone at room temperature to obtain (after solvent evaporation) a brown solid extract. The extract was further fractionated using methanol (90%) : hexane (1 : 1) liquid–liquid extraction as described by Demizu *et al* (1988) and Mitscher *et al* (1978) to obtain after the evaporation of the solvents, methanol (90%) and Hexane fractions. The evaluation of antioxidant activity of each fraction showed that the main antioxidant activity was found in methanol fraction. The methanol fractions were dried and reconstituted in 12% (v/v) aqueous ethanol.

2.5 *Evaluation of antioxidant activity*

Antioxidant activity of each plant extract was assayed by ferric reducing/antioxidant power (FRAP) assay as des-

cribed by Benzie and Strain (1999). Working FRAP (3·0 ml) reagent was mixed with 100 µl test sample or standard in a test tube; this was vortexed and the absorbance at 593 nm is read against a reagent blank at a predetermined time after sample-reagent mixing. For ease of use and reliability, aqueous ascorbic acid solutions at 100, 250, 500 and 1000 µM (equivalent to 200, 500, 1000 and 2000 µM FRAP) were used.

2.6 *Iron (Fe^{3+})-reducing activity*

The capacity of plant extracts to reduce Fe^{3+} was assessed by the method of Boyer *et al* (1988) with some modifications. The reaction mixtures comprised 50 mM sodium acetate (pH 5·5), 1 mM ferrozine, 100 µM $FeCl_3$ and 10 mg dried plant extract dissolved in a final volume of 1 ml distilled water. After adding the plant extract the reaction was started by addition of $FeCl_3$ (final volume of 1 ml) and the increase of absorbance at 562 nm was recorded after 3 min, using controls lacking the compound. A mixture of solutions without the compound were used as controls.

2.7 *Treatments*

Mice were anesthetized with ketamine and xylazine (200 and 2 mg/kg body wt. respectively) before the administration of KA, using the procedure as described in our previous work (Parihar *et al* 1997; Hemnani and Parihar 1999). Intra-hippocampal injection of KA (0·25 µg in volume of 0·5 µl) was administered with syringe fitted with a 26-gauge blunt-tipped needle into the dorsal hippocampus after exposing the skull along the midline. Controls were injected with normal saline instead of KA under the same experimental conditions. Doses of plant extracts given to experimental animals were based on their equivalent total antioxidant activity. Doses of 20 mg/kg body wt. of WS, 28 mg/kg body wt. of CP; 18 mg/kg body wt. of AV and their combinations were given to animals mixed with diet daily for 3 weeks before the induction of excitotoxicity. The diet was prepared by mixing freeze-dried plant extract with 10 g of standard mice food. All animals were housed individually in cages and checked daily to be certain that they consumed the full doses of plant extract.

2.8 *Brain tissue sampling and homogenization*

Twenty hours following KA injection, experimental mice were dissected and brain were removed and immersed in cold saline. The hippocampus was dissected from brain and pooled with other samples to get desired tissue for biochemical estimations. Tissue was homogenized at 0°C in 50 mM phosphate buffer (pH 7·4) for lipid peroxidation and glutathione peroxidase (GPx) assays; in 100 mM phosphate buffer (pH 7·4) containing 0·1% digitonin for protein carbonyl assay and in 0·1 M metaphosphoric acid containing 2 mM EDTA for GSH assay.

2.9 *Measurement of oxidative damage*

2.9a *Lipid peroxidation assay*: The extent of lipid peroxidation in brain homogenates was determined by measuring the release of thiobarbituric acid reactive substance (TBARS) in terms of malonaldehyde equivalent (MDA) using a molar extinction coefficient of $1{\cdot}56 \times 10^5$/min/cm as described by Ohkawa *et al* (1979). Briefly, the homogenate was centrifuged at 3000 *g* for 15 min and supernatant was used for assay. Samples of 0·1 ml homogenate were mixed with 0·2 ml of 8·1% SDS, 1·5 ml 20% glacial acetic acid and 1·5 ml of 0·8% thiobarbituric acid (TBA). Following these additions, tubes were mixed and heated at 95°C for 1 h on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5 ml mixture of n-butanol and pyridine (15 : 1). The mixture was centrifuged at 2200 *g* for 10 min. The amount of MDA formed was measured by the absorbance of upper organic layer at a wavelength of 532 nm in Perkin Elmer spectrophotometer using appropriate controls. The results are expressed as pmol MDA/mg protein.

2.9b *Protein carbonyl content assay*: The carbonyl content of brain homogenates was evaluated by the method of Levine *et al* (1990). Briefly, 100 µl of brain homogenates were incubated with 0·5 ml DNPH for 60 min. Subsequently, the protein was precipitated from the solution with the use of 20% TCA. The pellet was washed after centrifugation (3400 *g*) with ethyl acetate : ethanol (1 : 1 v/v) mixture three times to remove excess of DNPH. The final protein pellet was dissolved in 1·5 ml of 6 M guanidine hydrochloride. The carbonyl content was evaluated in a spectrophotometer at wavelength of 370 nm. A standard curve of bovine serum albumin (BSA) was included in each assay to determine linearity and measure the extent of derivatization. The results were presented in nmol/ mg protein.

2.10 *Measurement of antioxidants*

2.10a *GPx activity assay*: GPx activity (EC 1·11·1·9) was measured spectrophotometrically by a coupled enzyme procedure at 27°C monitoring loss of NADPH at 340 nm as described by Lawrence and Burk (1976). The homogenate was centrifuged at 4°C at 500 *g* for 15 min and the resulting supernatant at 10000 *g* for 20 min. The enzymatic reaction was conducted in 3 ml quartz cuvettes of

1 cm path length in a Perkin-Elmer spectrophotometer. The reaction mixture contained 50 mM phosphate buffer (pH 7·4), 4 mM NADPH, 1 unit glutathione reductase, 0·1 ml of supernatant sample and 0·7 mM H_2O_2 as substrate. Reactions were initiated by the addition of H_2O_2. Direct proportionality was seen with GPx enzyme activity over time and with sample concentration. GPx activity was estimated from the decrease of optical density at 340 nm due to NADPH oxidation between 2 and 4 min after the start of the reaction. The results are expressed as nmol NADPH oxidized/mg protein. One unit of the GPx activity is defined as the amount of enzyme necessary to catalyze 1 nmol NADPH/min/g wet weight at 27°C.

2.10b *Reduced GSH assay*: The GSH content of brain homogenates was quantitated by the method of Jollow *et al* (1974). Briefly, homogenate for GSH assay was centrifuged at 16000 *g* for 15 min at 4°C. The supernatant (0·5 ml) was added to 4 ml of ice-cold 0·1 mM solution of 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) in 0·1 M phosphate buffer (pH 8). The optical density was read at 412 nm in a spectrophotometer. A calibration curve was prepared using GSH as a standard.

2.11 *Total protein assay*

Total protein concentration of brain homogenates was determined by folin–phenol reaction as described by Lowry *et al* (1951). the bovine serum albumin (BSA) was used as a standard.

2.12 *Statistical analysis*

All data are expressed as mean ± SE. Statistical comparison was made relative to the appropriate control group by student's *t*-test and analysis of variance. The 0·05 level was selected as the point of minimal statistical significance in every comparison.

3. Results

3.1 *Excitotoxic neuronal damage*

The extent of excitotoxic neuronal damage was measured by assaying lipid peroxidation (figure 1) and protein carbonyl content (figure 2). The results demonstrated that hippocampal neurons are susceptible to oxidative attack by free radicals. After the administration of KA, a 3-fold increase in lipid peroxidation [211 ± 14 (control) to 647 ± 20 pmol/mg protein] and 4-fold increase in protein carbonyl [3·33 ± 0·44 (control) to 13·41 ± 1·23 nmol carbonyl/mg protein] were observed in comparison to control.

The antioxidant assay conducted after the administration of KA showed a link between neuronal oxidative insult and depletion of antioxidants in hippocampus region of brain. Both GPx activity and GSH content

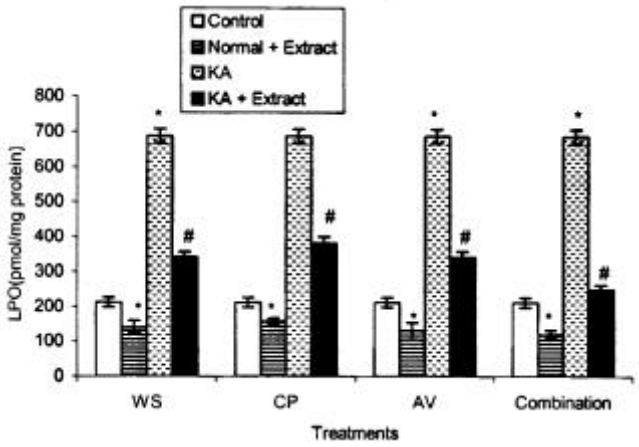

Figure 1. Effect of intra-hippocampal administration of KA and oral administration of ethanolic extract of WS, CP, AV and combination of WS + CP + AV on lipid peroxidation (LPO; pmol/mg protein) in hippocampus region of mice brain. Mice were pretreated with WS (20 mg/kg body wt.), CP (28 mg/kg body wt.), AV (18 mg/kg body wt.) and their combination for three weeks and then exposed to KA (0·25 µg in a volume of 0·5 µl). The animals were killed 20 h after KA injection. Values represent the mean ± SE.

*$P < 0·05$ significantly different from control. $^{\#}P < 0·05$ significantly different from KA treatment as determined by Student's *t*-test.

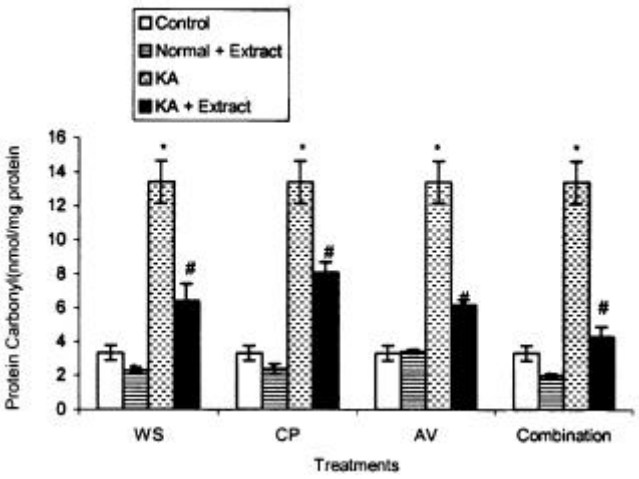

Figure 2. Effect of intra-hippocampal administration of KA and oral administration of ethanolic extract of WS, CP, AV and combination of WS + CP + AV on protein carbonyl (nmol/mg protein) in hippocampus region of mice brain. Mice were pretreated with WS (20 mg/kg body wt.), CP (28 mg/kg body wt.), AV (18 mg/kg body wt.) and their combination for three weeks and then exposed to KA (0·25 µg in a volume of 0·5 µl). The animals were killed 20 h after KA injection. Values represent the mean ± SE.

*$P < 0·05$ significantly different from control. $^{\#}P < 0·05$ significantly different from KA treatment as determined by Student's *t*-test.

declined significantly ($P < 0{\cdot}05$). GPx activity declined from $18{\cdot}41 \pm 2{\cdot}40$ (control) to $8{\cdot}21 \pm 1{\cdot}09$ nM NADPH oxidized/mg protein whereas GSH declined from $2{\cdot}30 \pm 0{\cdot}61$ to $0{\cdot}83 \pm 0{\cdot}14$ μmol/g wet tissue.

3.2 *Neuroprotective effect of plant extracts*

To evaluate potential neuroprotective effects of plant extracts against intra-hippocampal administration of KA, the animals were given oral dosage of aqueous extracts of either WS or CP or AV, and combined extracts of WS + CP + AV. The results showed a 2- to 3-fold reduction in lipid peroxidation (figure 1) and protein carbonyl content (figure 2) in the hippocampus of mice supplemented with these plant extracts. We found that pooling of plant extracts (WS + CP + AV) showed greater effects in reducing the lipid peroxidation and protein carbonyl in hippocampus than any of the plant extracts given singly. Supplementation with WS resulted in a significant ($P < 0{\cdot}05$) increase in GPx activity (figure 3) and GSH content (figure 4). GPx activity and GSH content also increased by supplementation with extracts of CP and AV, but the results were not significant ($P < 0{\cdot}05$). Thus we showed that pooling of plant extracts given to KA-induced mice produced a more prominent neuroprotective effect than the individual plant extracts.

We also examined the non-specific effect of plant extracts on normal mice. The results obtained demonstrated significant ($P < 0{\cdot}05$) neuroprotective effects of these plant extracts. Lipid peroxidation and protein carbonyl content were declined significantly ($P < 0{\cdot}05$). However, we obtained only a small increase in GPx activity and relatively no change in GSH content in the hippocampus of normal mice.

3.3 *Iron-reduction by plant extracts*

The results showed that all the three plant extracts tested in the present study (WS, CP, AV) reduce the Fe^{3+} as evidenced by an increase in absorbance at 562 nm. The WS and AV extracts were a very good reductants at pH 5·5 compared to CP (table 1).

4. Discussion

Researches have shown that the CNS may show an enhanced vulnerability to free radicals induced oxidative stress, since it is deficient in free radical protection and uses 20% of the total body oxygen (Olanow 1992) and this vulnerability may increase further in aging (Joseph *et al* 1996; Peng *et al* 2000; Aksenov *et al* 2001). Excitotoxicity is a major contributor to pathological cell death

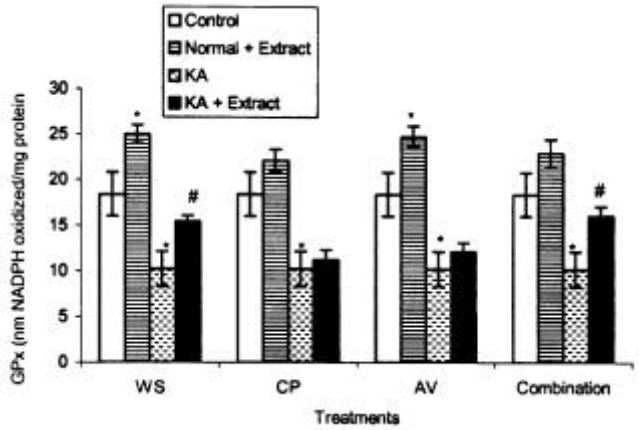

Figure 3. Effect of intra-hippocampal administration of KA and oral administration of ethanolic extract of WS, CP, AV and combination of WS + CP + AV on GPx (nM NADPH oxidized/mg protein) in hippocampus region of mice brain. Mice were pretreated with WS (20 mg/kg body wt.), CP (28 mg/kg body wt.), AV (18 mg/kg body wt.) and their combination for three weeks and then exposed to KA (0·25 μg in a volume of 0·5 μl). The animals were killed 20 h after KA injection. Values represent the mean ± SE.

*$P < 0{\cdot}05$ significantly different from control. $^{\#}P < 0{\cdot}05$ significantly different from KA treatment as determined by Student's *t*-test.

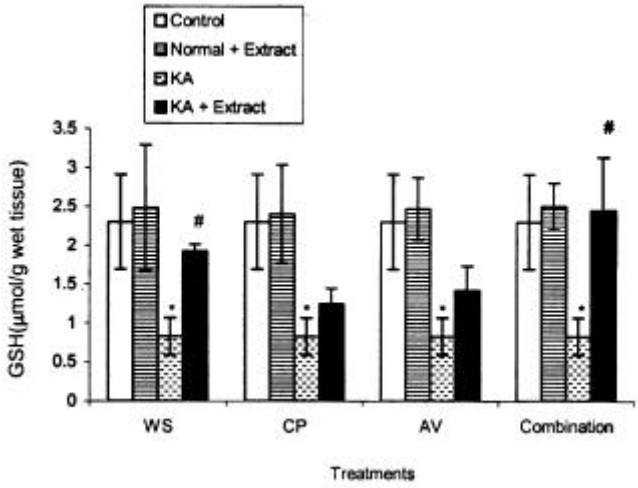

Figure 4. Effect of intra-hippocampal administration of KA and oral administration of ethanolic extract of WS, CP, AV and combination of WS + CP + AV on reduced GSH (μmol/g wet tissue) in hippocampus region of mice brain. Mice were pretreated with WS (20 mg/kg body wt.), CP (28 mg/kg body wt.), AV (18 mg/kg body wt.) and their combination for three weeks and then exposed to KA (0·25 μg in a volume of 0·5 μl). The animals were killed 20 h after KA injection. Values represent the mean ± SE.

*$P < 0{\cdot}05$ significantly different from control. $^{\#}P < 0{\cdot}05$ significantly different from KA treatment as determined by Student's *t*-test.

Table 1. Iron-reducing activity of plant extracts*.

Plant extract	Absorbance 562 nm at pH 5·5
Control (no compound)	0
W. somnifera	1·212 ± 0·42
C. pleuricauas	0·855 ± 0·19
A. vera	1·110 ± 0·31

*, Fe^{3+} reduction was measured by the ferrozine assay at pH 5·5. Activity is expressed as the increase in absorbance at 562 nm after 3 min and values represent means of 3 independent replicates.

within the nervous system and appears to be mediated by reactive oxygen species (Beal 1997; Coyle and Puttfarcken 1993; Law *et al* 2001). Accumulation of free radical damage as the cause of aging is supported by studies that show increased oxidatively induced protein and lipid damage in pathological conditions of accelerated aging in human and mice (Stadtman 1992; Bondy 1997). A particularly important consequence of free radical damage in many cells, is the peroxidation of polyunsaturated fatty acids (PUFA), which results in the formation of lipid peroxides and aldehydes (Cheeseman and Slater 1993; Murphy *et al* 1989), and carbonyl modifications of proteins which may affect a variety of cellular functions involving proteins: namely; receptors, signal transduction mechanisms, transport systems and enzymes.

In the model of KA-induced excitotoxic neuronal death *in vivo* we observed a pronounced increase in hippocampal neuronal degeneration marked by a sharp increase in lipid peroxidation and protein carbonyl after KA injection. The demise of hippocampal neurons occurring as a result of excitotoxicity suggests the role of increased free radical production.

Increase in oxidative stress would also contribute to progressive impairment of the antioxidant reserves of the brain. GSH is an important antioxidant that limits oxidative damage caused by reactive oxygen species (ROS), many of which are generated as a consequence of normal metabolic activity. It acts as both a nucleophilic scavenger of toxic compounds and as a substrate in the GPx-mediated destruction of hydroperoxides (Meister 1983). The depletion of GSH would be expected to compromise this pathway and may thereby allow H_2O_2 to accumulate to toxic levels. Brain tissues are especially vulnerable to this effect since antioxidative enzymes are relatively deficient in the brain (Marklund *et al* 1982; Martilla *et al* 1988) In particular, the brain has a relatively low H_2O_2 metabolizing enzyme catalase (Benzi and Moretti 1995). A vicious cycle of increasing oxidative stress may slowly damage neurons over a period of years, eventually leading to the neuronal cell death characteristic of the sporadic, age-related neurodegenerative diseases. Not unexpectedly, the increase in excitotoxicity-induced free radical load observed under KA treatment contributed to a decline in GPx activity and GSH content in hippocampus. This decline in GPx activity and GSH content might represent an index of cellular damage prior to the neuronal death. Regardless of the compounds used to deplete GSH, there is general agreement that GSH must be depleted below a certain critical threshold in order for large increases in lipid peroxidation and necrotic cell death to occur (Tirmenstein *et al* 2000).

Several studies have suggested that these increases in oxidative stress vulnerability and the resulting neuronal loss can be reduced through dietary supplementation of plant extracts that prevent brain atrophy as well as learning and memory impairments (Kanowski *et al* 1996; Moriguchi *et al* 1997; Nishiyama *et al* 1997). In addition, to the effects of phytochemicals in plants protecting brain cells, flavonoids too can increase membrane fluidity (Halder and Bhaduri 1998; Ramassamy *et al* 1993; Stoll *et al* 1996) and decrease membrane rigidity (Joseph *et al* 1995). Findings from our laboratory also suggest that plant extracts which are high in total antioxidant activity as assessed via FRAP assay, prevented the onset of the deleterious effects of KA-induced excitotoxicity in hippocampus. With respect to the individual functional neuronal indices, the mice treated with WS showed the greatest reduction in neuronal cell damage. There was a 2- to 3-fold decline in lipid peroxidation and protein carbonyl content. The GPx activity and GSH content also increased significantly ($P < 0·05$) by supplementation of WS and combination of WS, CP and AV. Furthermore, it appears that combination of plant extracts high in phytonutrient antioxidants are more effective in attenuating oxidative damage in brain compared to the plant extracts given singly. The combination resulted in a 3-fold decline in lipid peroxidation and protein carbonyl while increasing GPx activity and GSH content in hippocampus significantly ($P < 0·05$). The data obtained in this study clearly showed that there were no significant differences in GPx activity and GSH content as a function of neuroprotection by CP and AV despite reductions in neuronal damage in hippocampus. The antioxidants levels as determined by FRAP assay were almost similar in the plant extracts, however, the other properties may act in concert with them to produce these differential effects. This would suggest that there might indeed be properties, in addition to antioxidant characteristics, that are promoted by phytochemicals present in plant extracts involved in increasing excitotoxic neuronal damage.

Since the mammalian hippocampus plays a pivotal role in a diverse set of cognitive functions, such as novelty detection and memory (Bardgett and Henry 1999; Culmsee *et al* 2001; Suzuki and Clayton 2000), the antioxida-

tive properties of plant extracts studied in this work may play an important role in improving cognitive function against excitotoxicity. The present work clearly showed that plant extracts have the capacity to reduce the Fe^{3+} which may restrict the interaction of the metal ion with membrane lipids, thus avoiding oxidative damage to membrane lipids or proteins. The strong Fe^{3+} reducing activities of the plant extracts and the decline in oxidative damage in hippocampus of KA-induced mice indicate that the plant extracts have a primary role as antioxidants by destroying free radicals and sequestering Fe in a safe catalytically inactive form.

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