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Antioxidative Effect of *Trichosanthes tricuspidata* Root Extract on Sildenafil Induced Migraine in Albino Mice

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ABSTRACT

Migraine is considered a neurovascular disease involving the dilatation of cerebral arteries. Because of their dual role in modulating neuronal and vascular events, neuropeptides have been implied to be of importance in migraine pathophysiology. It is a highly prevalent disorder which manifests clinically as moderate to severe or severe headache attacks with frequent frontotemporal unilateral location and associated symptoms. The disability of migraine results in considerable economic and social losses .Nitric oxide, a short lived vasodilator and weak oxygen radical has been implicated in the genesis of migraine headaches. Hence the role of all enzymic and non enzymic antioxidants becomes in evitable in the treatment of migraine. *Trichosanthes tricuspidata*'s (red apple gourd) antioxidant effect was tested over Sildenafil induced migraine in female albino mice. The levels of antioxidant enzymes such as super oxide dismutase (SOD), lipid peroxidase(LPO), catalase(CAT) and glutahione peroxidase(GPx) were more promising in the treatment group fed with the root extract of Trichosanthes tricuspidata.

Keywords: nitric oxide, oxygen radical, Sildenafil, stress.

INTRODUCTION

Migraine is a neurological disorder that is more prevalent among females than males. Nearly 27million females and 10 million males were affected by this in US (1). Though there are many causes for migraine genesis, the foremost reason is the increased stress condition. The pain is pulsating and/or pressure-type, usually associated with nausea, photophobia, phonophobia and osmophobia (2). Its attacks promote disability and generally worsen with physical activities. The duration may last from 4 to 72 hours when not treated or treated ineffectively. The headache frequency is variable and some patients may present it on a weekly basis while others will have it less than once a month. There are four different phases of Migraine attacks: (i) prodromic phase with premonitory symptoms, (ii) aura phase with transient neurological symptoms and signs, (iii) headache phase with associated features and (iv) recovery or postdromic phase frequently associated with resting and sleeping. Only the headache phase can be treated and despite the advances in understanding migraine, considerable uncertainty surrounding an effective and definitive way of treating the attacks remains (3).

Several drug options and different formulations are available to treat migraine acutely. The choice of a specific medication type depends on individual characteristics such as headache intensity, speed of onset of action, presence of associated symptoms, the degree of incapacitation, and the patient's response (4).Still the search for remedies doesn't end, several phytochemicals and phyto drugs were tested for its efficacy against migraine. The plant *Trichosanthes*

tricuspidata, commonly known as red apple gourd, a vine, under the family *Cucurbitaceae* is used in the indigenous medicinal system of India for the treatment of several stress related problems, ophthalmic disorders, epileptic conditions(5) . Though it is a poisonous medicinal plant, it contains many therapeutically important active principles like trichonin, santholin, cycloeucaienol, β – sitosterol, cycloartane, cucurbitane and α- spinasterol (6, 7). Hence the root extract of *Trichosanthes tricuspidata* was taken and administered orally in a dose that was not toxic (LD_{50}) to the female albino mice with experimentally induced migraine by Sildenafil.

The exact pathophysiology of migraine attack was still not known. But it was found that NO may initiate the migraine attack but may also be involved in propagating pain throughout the attack. Also, there is evidence of crosstalk between NO and the neurotransmitter calcitonin gene-related peptide (CGRP), which has been found to be released during migraine attacks(8) at the level of cyclic nucleotides, and the vasodilating effects of NO and CGRP are suggested to interact at this level(9,10,11). The main effect of NO is to activate intracellular soluble guanylate cyclase and thus catalyse the formation of cyclic guanosine monophosphate (cGMP). However, NO has a variety of other actions, such as binding to ion channels, activating phosphokinases, and possibly activating nociceptive nerve fibres directly via the formation of free radicals such as hydroxyl ions(12). Thus to counteract the ill effects produced by NO synthesis the antioxidant defense system must be triggered on and supplementation of antioxidant rich substances might reduce the risk of getting migraine and its aftermath effects very much. The migraine headache can be artificially induced by sildenafill (Viagraâ), a highly selective inhibitor of phosphodiesterase 5 (PDE5), which is the major enzyme responsible for the breakdown of cGMP(13). Inhibition of this enzyme results in accumulation of cGMP, and the effect of Sildenafil, therefore mimics one of the effects of NO (activation of soluble guanylate cyclase and increased cGMP formation) but not its other effects (14).

MATERIALS AND METHODS

Preparation of *Trichosanthes tricuspidata* root extract

50 grams of *Trichosanthes tricuspidata* root was taken shade dried, powdered well. They were soaked with adequate water, and kept at room temperature for 2 days. It is then homogenized, filtered and made up to 100ml; the liquid part is stored at 4°C.

Female albino mice weighing about 50 – 75 grams were used as experimental animals. The animal experiments were carried out in accordance with the rules of the institutional animal ethical committee. The animals were acclimatized in laboratory condition for 10 days and were fed with normal rodent diet (pellet diet), water was given *at libitum*. After complete acclimatization the animals were primarily grouped into 4 groups, each containing 6 animals. *Group I* -Served as normal *control, Group II*- Served as an experimental control (administered with Sildenafil 500mg/kg of body weight during the last three days of the treatment), *Group III*- contains animals administered with the root extract alone orally (2ml/day) for 30 days, *Group IV*- contain animals fed with root extract of *Trichosanthes tricuspidata* and migraine induced by three doses of Sildenafil (500mg/kg of body weight) during the last three days of the treatment. The extract was administered orally for 30 days.

At the end of 30days the animals were fasted overnight, weighed and sacrificed with mild ether anesthesia. Blood was collected, serum separated and stored for biochemical estimations. Analysis of serum Protein (15), SOD (16), CAT (17), GPx (18) and LPO (19) were carried out. The results were tabulated and statistical analysis was performed using Student's't' tests (20).

RESULTS AND DISCUSSION

Antioxidant compounds in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce risk for chronic diseases including cancer, heart disease and several complications (21). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavanoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (22). Induction of stress is the first and foremost cause for the onset of migraine (23). Migraine headache is a public health problem of enormous scope that has an impact both on the individual sufferer and on the society. Tension-type headache is more common in women, with gender ratios ranging from 1.04 to 1.4. Prevalence peaks between the ages of 20 and 50 years (24). Tension-type headaches often interfere with the activities of daily living. (25, 26).

The pathophysiology of migraine is not well established but the generation of free radicals and the degenerative effects rendered by them are considered to be more pronounced for the onset of migraine (27, 28, 29, 30, and 31). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are the antioxidant enzymes which are known to have critical importance in antioxidant system. These enzymes affect free radicals

Table 1 Effect of the ethanolic extract of *Trichosanthes tricuspidata* root, on Sildenafil induced migraine in albino mice

Groups	Catalase (I.U. mg protein$^{-1)}$	Superoxide dismutase (I.U. mg protein^{-1})	Glutathione peroxidase (nmol NADPH consumed min^{-1} mg protein $^{-1}$)	Lipid peroxidase (nmol malondialdehyde mg protein^{-1} 30 min^{-1})
Group I	35.40 ± 0.99	2.90 ± 0.12	10.02 ± 0.27	16.05 ± 0.56
Group II	23.19 ± 1.24	1.80 ± 0.08	6.88 ± 0.72	9.65 ± 0.22
Group III	35.56 ± 0.92	2.73 ± 0.14	9.56 ± 0.23	14.32 ± 0.68
Group IV	32.01 ± 0.12**	2.59 ± 0.70**	9.49 ± 0.81**	14.26 ± 0.16**

Group I = Normal control, Group II = Sildenafil induced, Group III = Tricosanthes tricuspidata root extract alone treated, Group IV = Sildenafil induced = Tricosanthes tricuspidata root extract treated. Group III were compared with Group I and Group IV were compared with Group II. Values are expressed as mean ± Standard Deviation for 6 animals in each group.

*= Significant when compared to group II

**= Not significant when compared to group II (P = <0.05)

in metabolic pathways in different places (32, 33, 34). They have an important role in clearance of free radicals against tissue defect caused by these radicals. The role of antioxidant enzymes in migraine patho physiology has been reported (35,36,37). Hence the parts of *Tricosanthes tricuspidata* plant were tested for its efficacy against migraine induced by Sildenafil citrate (Viagra). This *Tricosanthes tricuspidata* root was in use for neural related problems in the indigenous medicinal systems of India and proved to be rich in antioxidants (38).

The serum total protein was analyzed by Lowry's *et al,* method. The protein value was found to be increased in the serum of animals belonging to migraine induced groups and it was more pronounced in G II migraine induced animals on treatment with the *Tricosanthes tricuspidata* root extract, the reversal of the concentration was identified. Significant decrease was noted in migraine induced animals treated with ethanolic extract of the root. This increase in the total serum protein content might be attributed by the draining of degenerated tissue protein in the circulatory fluid (39).

SOD, CAT, and GR are known to be inactivated in vitro by H_2O_2, O_2.-, and OH, respectively. SOD and CAT are major antioxidant defense components that primarily catalyze the conversion of superoxide radical O_2 - to H_2O_2 (SOD) and decomposition of H_2O_2 to H_2O (CAT). H_2O_2 is normally detoxified in cells by either CAT and or GPx (Glutathione peroxidase). GPx catalyzes the reduction of H_2O_2 by reduced glutathione (GSH). GSH is readily oxidised to glutathione disulfide (GSSG) by the GPx reaction (40). GSSG can be reduced by NADPH-dependant reaction catalysed by glutathione reductase. A decrease in SOD, CAT and GPx activity with the migraine induction probably results in accumulation of O_2.- and H_2O_2 which react with metal ions to promote additional radical generation, with the release of the particularly reactive hydroxyl radical. Hydroxyl radicals reacts with lipids, DNA and proteins, caused a loss of cell integrity, enzyme function and genomic stability. The migraine induced by sildenafil resulted in the decrease of the enzymes levels of SOD(Cu-Zn SOD),CAT , GPx, GST and GSH (41,42). On treatment with the *Tricosanthes tricuspidata* root extract the levels increased significantly and the effect was greater in migraine induced animals which received nearly 30 days of drug treatment. The free radicals generated as a result of migraine induction to propagate a chain reaction, leading to lipid peroxidation in cellular membranes, destruction of Ca^{2+} homeostasis that induces neuronal cell injury and finally results in cell Death (43). The groups induced with migraine and treated with *Tricosanthes tricuspidata* root extract showed increase in the antioxidant enzymes concentration, when compared to the experimental group. The increase in the antioxidant enzymes in these treated groups represents the decrease in the generation of free radicals which might be attributed by the antioxidant property of the plant parts (44).

Sildenafil induced migraine in mice has been established here by noting the low activities of SOD, CAT, GST- important antioxidant enzymes, which is consistent with the observation of others (45, 46). The decrease in antioxidant enzyme activities due to migraine might be due to their use against the free radicals destruction and or their inhibition by free radical species (47). It is well established that SOD activity is inhibited by hydrogen peroxide that reduced Cu^{+2} to Cu^{+1} in SOD (44).

The reduction of hydrogen peroxide is catalyzed by CAT that protects the tissues from highly reactive hydroxyl radicals (48). Reduction of hydrogen peroxide and hydro peroxides to non-toxic products are catalyzed by GST and peroxidase. From the results obtained after treating the migraine of the animals induced by the sildenafil with the root extract of *Tricosanthes tricuspidata* showed that the treatment had prevented the free radical toxicity production and related tissue degeneration .This could be attributed by the antioxidant nature of the plant.

REFERENCES

1. Pearce,J.M.S. *Headache. Neurological Management* series. *Journal of Neurology Neurosurgery and Psychiatry*. **57**:134-144 (1994).

2. Buchholz, D. *Heal your headache: The 1-2-3 Program*, New York: Workman Publishing, ISBN 0-7611-2566-3 (2002).
3. Olesen J, Peer TF-Hansen, K. Michael AW. The Headaches. Philadelphia: Lippincott-Williams andWilkins,227-33 (2000).
4. Silberstein, Stephen D.; Lipton, Richard B.; Goadsby, Peter J. *Headache in Clinical Practice* 2 Edition. Andover: Thomson Publishing Services. (2002).
5. Singh HP. National perspective on development of medicinal and aromatic plants. Technical report. AgriWatch. ext-link-type="uri" xmlns:xlink="http://www.w3.org/1999/xlink" xlink:href="http://www.agriwatch.com">http://www.agriwatch.com, (2001).
6. Mai le P, Guenard D, Franck M, Van TM, Gaspard C and Sevenet T, New cytotoxic cucurbitacins from the pericarps of *Trichosanthes tricuspidata* fruits. *Natural Product Letters* **16**(1):15-19 (1994).
7. Kasai R, Sasaki A, Hashimoto T, Ohtani K and Yamasaki K, Cycloartane glycosides from *Trichosanthes tricuspidata*. *Phytochemistry* **51**:803-808 (1999).
8. Goadsby PJ, Lipton RB, Ferrari MD. Migraine: current understanding and treatment. *N Engl J Med*. **346**:257-70 (2002).
9. Gray DW, Marshall I. Human alpha-calcitonin gene-related peptide stimulates adenylate cyclase and guanylate cyclase and relaxes rat thoracic aorta by releasing nitric oxide. *Br J Pharmacol*. **107**: 1–696 (1992).
10. Wei EP, Moskowitz MA, Boccalini P, Kontos HA. Calcitonin gene-related peptide mediates nitroglycerin and sodium nitroprusside-induced vasodilation in feline cerebral arterioles. *Circ Res*, **70:** 1313–9 (1992).
11. Pelligrino DA, Wang Q. Cyclic nucleotide crosstalk and the regulation of cerebral vasodilation. (Review). *Prog Neurobiol*. **56**: 1–18 (1998).
12. Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. (Review). *Annu Rev Physiol*. **57**: 683–706 (1995).
13. Farooq Muhammad U, Naravetla Bharath, Moore Philip W., Majid Arshad, Gupta Rishi; Kassab Mounzer Y. MD, MA.Role of Sildenafil in Neurological Disorders. *Clinical Neuropharmacology* **31**(6):353-362 (2008).
14. Schultheiss D, Muller SV, Nager W, Stief CG, Schlote N, Jonas U, et al. Central effects of sildenafil (Viagra) on auditory selective attention and verbal recognition memory in humans: a study with event-related brain potentials. *World J Urol*. **19**: 46–50 (2001).
15. Lowry OH, Rosebrough NJ, Farr AC, Randall RS, Protein measurements with the Folin-Phenol reagent. *Journal of Biological Chemistry* **193**:65-75 (1951).
16. Suttle NF. Analysis of super oxide dismutase. Vet Rec. **119**:519-22 (1986).
17. Bergmeyer H, Gawehn K, Grasse M, *Methods of enzyme analysis: Enzymes as biochemical reagents*. (In: Bergmeyer HV, editor), Academic Press, New York, pp. 438-448 (1974).
18. Rotruck,JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Haekstra WG, Selenium: biochemical role as component of glutathione peroxidase. *Science* **179**: 588-590 (1973).
19. Donnan SK. The thiobarbituric acid test applied to tissues from rats treated in various way. *J Biol Chem*. **182**:415-9 (1950).
20. Bennett C A and Franklin N L. Statistical analysis in Chemistry and chemical industry (John Willey and sons, Inc, New York), 133 (1967).
21. Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk J Biol* **30**: 177-183 (2006).
22. Bors W, Saran M, Elstner EF. Screening for plant anti-oxidants. In: Linskens HF, Jackson JF. eds. Modern Methods of Plant Analysis-Plant Toxin Analysis-New Series, Vol **13**. Springer, Berlin; pp. 277-295 (1992).
23. Silberstein SD, Saper JR, Freitag F. Migraine: Diagnosis and treatment. In: Silberstein SD, Lipton RB, Dalessio DJ, eds. Wolff's headache and other head pain. 7th ed. New York: Oxford University Press:121-237 (2001).
24. Edmeads J, Mackell JA. *The economic impact of migraine: an analysis of direct and indirect costs. Headache*. **42**:501-509 (2002).
25. Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. *Cephalalgia*;(suppl 7):1-96 (1988).
26. Olesen J, Peer TF-Hansen, Michael AW. K. The Headaches. Philadelphia: Lippincott-Williams and Wilkins; 227-33 (2000).
27. Hassinger HJ, Semenchuk EM, O'Brien WH. Cardiovascular responses to pain and stress in migraine. *Headache*, **39**:605-15 (1999).
28. Kowa H, Yasui K, Takeshima T, Urakami K, Sakai F, Nakashima K. The homozygous C677T mutation in the methylentetrahydrofolate reductase gene is a genetic risk factor for migraine. *Am J Med Genet* **96**:762-4 (2000).
29. Tozzi E, Tozzi-Ciancarelli MG, Di Massimo C, Mascioli A, Angelini R, de Matteis F. Platelet responsiveness in migrainous children during headache-free period. *Headache* **36**:95-9 (1996).
30. Schlesinger RH. Homocysteine levels in migraine patients. *Cephalalgia* **17**:46 (1997).
31. Goadsby PJ, Lipton RB, Ferrari MD. Migraine: current understanding and treatment. *N Engl J Med* **346**:257-70 (2002).
32. Fahr S, Cohen G. The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol*. **32**:804-12 (1992).
33. Clemens MR, Walker HD. Lipid peroxidation in erythrocytes. *Chem Phys Lipids* **45**:251-68 (1987).
34. Halliwel B. Oxidant and human disease: some new concepts. *Faseb J*. **1**:358-64 (1987).
35. Shimomura T, Kowa H, Nahano T, Kitano A, Marukawa H, Urakami K, Takahashi K. Platelet superoxide dismutase in migraine and tension- type headache. *Cephalalgia*;**14**: 215-8 (1994).
36. Ikeda Y, Jimbo H, Shimazu M, Satoh K. Sumatriptan scavenges superoxide, hydroxyl, and nitric oxide radicals: *in vitro* electron spin resonance study. *Headache*, **42**:888-92 (2002).
37. Choudhuri R, Cui I, Yong C. Cortical spreading depression and gene regulation: relevance to migraine. *Ann Neurol*. **51**:499-506 (2002).
38. Kanchanapoom T, Ryoji K and Yamasaki K, Cucurbitane, hexanorcucurbitane and octanorcucurbitane glycosides from fruits of *Trichosanthes tricuspidata*. *Phytochemistry* **59**:215-228 (2002).
39. Verotti A, Basciani F, Trotta D, Pomilio MP, Morgese G, Chiarelli F. Serum copper, zinc, selenium, glutathione peroxidase and superoxide dismutase levels in epileptic children before and after 1 year of sodium valproate and carbamazepine therapies. *Epilepsy Res*. **48**:71-5 (2002).
40. Krause RJ, Gauther HE. Reaction of cyanide with glutathione peroxidase. *Biochem Biophys Res Com*. **96**:1116-22 (1980).
41. Percy ME, Dalton AJ, Markovic VD, Mclachlan DR, Hummel JT, Rusk AC, Andrews DF. Red cell superoxide dismutase, glutathione peroxidase and catalase in Down syndrome patients with and without manifestations of Alzheimer's disease. *Am J Med Genet*. **35**:459-67 (1990).
42. Jackson G, Benjamin N, Jackson N, Allen MJ. Effects of sildenafil citrate on human hemodynamics. *Am J Cardiol*. **83**: 13–20 (1999).
43. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. **70**:158-69 (1967).
44. Kitajima J, Mukai A, Masuda Y and Tanaka Y, Studies on the constituents of *Trichosanthes* root. III. Constituents of roots of *Trichosanthes bracteata* Voight. *Yakugaku Zasshi* **109**(4):265-270 (1989).
45. Saggu H, Cooksey J, Dexter D, Wells FR, Lees A, Jenner P, Marsden CD. A selective increase in particulate superoxide dismutase activity in Parkinsonian substantia nigra. *J Neurochem*. **53**:692-7 (1989).
46. Percy ME, Dalton AJ, Markovic VD, Mclachlan DR, Hummel JT, Rusk AC, Andrews DF. Red cell superoxide dismutase, glutathione peroxidase and catalase in Down syndrome patients with and without manifestations of Alzheimer's disease. *Am J Med Genet*. **35**:459-67 (1990).
47. Olsen TS, Lassen NA. Blood and vascular reactivity during attacks of classic migraine limitations of the Xe-133 intraarterial technique. *Headache* **29**:15-20 (1989)
48. Lunec J. Free radicals: their involvement in disease processes. *Ann Clin Biochem*. **27**:173-82 (1990).