

EFFECT OF EXTRACT OF *BENINCASA HISPIDA* ON OXIDATIVE STRESS IN RATS WITH INDOMETHACIN INDUCED GASTRIC ULCERS

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Abstract : This study was undertaken to determine the healing of ulcers induced by indomethacin due to antioxidant role of fruit extract of *Benincasa hispida* (Ashgourd) on ulcers in rats. Malondialdehyde (MDA) in RBC and antral homogenate was determined to measure tissue oxidation. Superoxide dismutase (SOD) in RBC and antral homogenate, plasma and homogenate vitamin C were estimated as measures of antioxidant defense. On induction of gastric ulcer, there was significant increase in SOD in RBC and homogenate levels and vitamin C in plasma. There was an apparent decrease in ulcer index in animals treated with fruit extract. There was significant decrease in MDA with concomitant decrease in SOD and vitamin C levels in the treated rats when compared to those not treated with fruit extract. *Benincasa hispida* has been shown to contain certain active principles like terpenes, flavanoid C-glycosides and sterols which have antioxidant effects. These probably inhibit gastric mucosal injury by scavenging the free radicals and repress production of SOD and vitamin C in these rats.

Key words : benincasa hispida ulcer SOD vitamin C MDA

INTRODUCTION

Benincasa hispida (Ash gourd, Family: Cucurbitaceae) is a commonly used vegetable, which has found mention in 'Charaka Samhita' for its medicinal properties. It is the main ingredient in 'Kusumanda Lehyam' used in Ayurvedic system of medicine as a rejuvenating agent and in the treatment of nervous disorders. Fruit has also been used in India to treat disorders of the GI tract,

respiratory tract, urinary tract and diabetes mellitus (1, 2). It is known to render protection against histamine induced bronchospasm (3). A multitude of studies have shown that the antioxidant status of an individual was compromised in most of these disorders.

Previous studies have shown the healing effect of the fruit extract of *Benincasa hispida* on experimentally induced ulcers in rats (4).

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Oxidative stress is believed to initiate and aggravate many diseases including peptic ulcers (5).

In the present study, we have studied the healing effect of fruit extract of *Benincasa hispida* on indomethacin induced ulcers in rats. The effect of indomethacin and fruit extract treatment on antioxidant status of these animals were assessed by determining MDA levels and antioxidants viz. vitamin C and SOD.

MATERIAL AND METHODS

Preparation of fruit extract: Ash gourd was purchased from the local market. The pulp of ash gourd was removed, homogenised and filtered using cheese cloth. The extract thus obtained was fed to the rats.

Animals: Male albino rats weighing around 200 g were obtained from animal house, Kasturba Medical College, Mangalore. They were maintained for a week before the experiment. Food and water were given ad libitum during this time. 21 rats were divided into three groups of 7 each.

Group I : Normal, fed with gum acacia.

Group II : Control, fed with indomethacin dissolved in gum acacia only.

Group III: Test, fed with indomethacin in gum acacia followed by fruit extract.

Induction of gastric ulcers: Gastric ulcers were induced in control and test groups of rats, previously fasted for 24 h by

administering indomethacin (30 mg/kg, body weight suspended in 2% aqueous gum acacia) to the rats after 12 h starvation, as a single dose, orally, for 6 days.

Six h after the administration of the drug, fruit extract (1 ml/kg, body weight) was given to the animals of the test group and 2% gum acacia to the animals of control group. On the 7th day all the animals were sacrificed by administering ether. Blood was collected in heparinized syringe by cardiac puncture. Stomach was dissected out, observed for ulcers and was evaluated according to Shays method for ulcer index (6) Malondialdehyde (MDA) (7), superoxide dismutase (SOD) (8) were estimated in RBC suspension and supernatant of stomach tissue homogenate (10% in cold 2 mmol phosphate buffer, pH 7.2). Vitamin C (9) was determined in plasma and tissue homogenate. Proteins (10) in tissue homogenate and hemoglobin in RBC suspension by cyanmethemoglobin method were also estimated.

Statistical analysis was done using Mann-Whitney U test.

RESULTS

In this study, there was an apparent decrease in ulcer index when the rats were treated with fruit extract after induction of ulcer by indomethacin.

There was an apparent increase in RBC and tissue extract MDA levels in controls when compared with normal group and there was a significant decrease in RBC and tissue MDA levels in the test when compared to controls.

In the present study, there was a statistically significant increase in SOD levels on induction of ulcers with indomethacin both in RBC and tissue extract compared to normal. However, the decrease in level of SOD in the test group was statistically significant both in the homogenate and RBCs compared to control. In fact, the decrease in SOD levels in test group as compared to the normal was also statistically significant. There was an apparent decrease in vitamin C level in antral homogenate in controls as compared to normals and a statistically significant decrease in test as compared to control. There was a statistically significant increase in plasma vitamin C in controls compared to normals and a statistically significant decrease in test as compared to control.

DISCUSSION

Peptic ulcer and gastritis have multipathogenic factors. There is substantial evidence that oxygen derived free radicals play an important role in the pathogenesis of various diseases, including gastric ulcers (11, 12). In addition, involvement of oxygen derived free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radicals are well established in the injury of gastric mucosa and in other models of mucosal damage induced by nonsteroidal antiinflammatory drugs and *H. Pylori* (13) ethanol (14) and feeding restriction stress (15).

It has been reported previously, in gastric carcinoma patients that there was a decrease in ulcer index (5). Even in the present study, there was an apparent decrease in the ulcer index when the rats

were treated with the fruit extract after induction of ulcer by indomethacin (Table I).

The fact that there was an increase in MDA levels in controls compared with normal and its decrease when test value was compared with controls, indicates that there was an increase in oxidative tissue damage on induction of ulcer with indomethacin, which decreased with the administration of the fruit extract.

TABLE I: Comparison of values of the different parameters between normal, test and control rats.

Parameters	Normal	Test (Indome- thacin+ Fruit extract)	Control (Indome- thacin)
RBC:			
MDA (nmol/ dl RBC)	0.74 ± 0.098	0.64 ± 0.015 ³	0.79 ± 0.156
SOD (U/g Hb)	4122.71 ± 2809	2742.71 ± 1094 ^{2,b}	8641 ± 3820**
PLASMA			
Vitamin C (mg/dl)	680.33 ± 520.86	774.35 ± 210.64 ¹	984.60 ± 448.34**
HOMOGENATE :			
MDA (nmol/g tissue)	9.13 ± 0.86	4.83 ± 0.20 ²	9.15 ± 1.3
SOD (U/mg total protein)	45455 ± 1855	37536 ± 26586 ^{a,2}	66168 ± 13546*
Vitamin C (mg/dl)	21.7 ± 11.01	6.54 ± 2.7 ³	19.87 ± 5.65
Ulcer index		0.45 ± 0.19	0.62 ± 0.19

N = 7

KEY: *, a, 1 are significant; P<0.05; **, b, 2 are highly significant; P<0.002; ***, c, 3 are very highly significant; P<0.001; *, **, ***: Normal vs Control; a, b, c: Normal vs Test; 1, 2, 3: Control vs Test.

There are evidences that a number of plants and plant products like *Ocimum sanctum* (16), piper betel leaves (17), rhizome of *Zingiber officinale* (18), rhizome of *Picrorhiza kunooa* (19), *Phyllanthus emblica* (20) have potent antiulcerogenic properties and are known to heal ulcers. Some scientists (21) are of the opinion that all fruits and vegetables contain an abundance of terpenoids and phenolic substances which act as antioxidants and afford protection when used in treatment of certain chronic diseases such as heart diseases and cancer where oxidative stress has been implicated. Phytochemical screening of *Benincasa hispida* fruit extract has shown the presence of four terpenes, two sterols and a flavanoid C-glycoside (22). It may be possible that these compounds are responsible for the antiulcerogenic and antioxidant role of *Benincasa hispida*. These compounds probably inhibit gastric mucosal injury by scavenging the indomethacin generated oxygen metabolites.

The statistically significant increase in SOD levels on induction of ulcers with indomethacin both in RBC and tissue extract indicates that this enzyme was induced to counteract the oxidant effect of indomethacin. However, the significant decrease in level of SOD after administration

of fruit extract in the homogenate and in RBC, probably indicates the presence of some antioxidants in the fruit extract which represses the production of the enzyme.

This fact is justified by an earlier study (23) where three flavone C – glucosides have been identified in *Atractylodes japonica* which were shown to have hydroxyl and superoxide anion radical scavenging activities and decreased the formation of MDA and TBA reactive substances, which are intermediates of lipid peroxidation.

The decrease in hydrophilic antioxidant, vitamin C level in antral homogenate indicates sparing effect on vitamin C and SOD by the antioxidants present in the extract of *Benincasa hispida*. An earlier study (24) has demonstrated the efficacy of mono, di and γ -terpenes as antioxidants and these terpenes may act synergistically with hydrophilic antioxidants in their action.

The decrease in antioxidant parameters in the study could be due to the sparing effect exerted by other antioxidants present in the extract of *Benincasa hispida*. Further studies may be required to determine as to which of the active principles of *Benincasa hispida* is responsible for the antioxidant activity.

REFERENCES

1. Aslokar LV, Kakkar KK, Chakre OJ. Glossary, Indian medicinal plants with active principles. Part I, first edition, New Delhi, CSIR 1992; p.119.
2. Sivarajan VV, Balachandran I, eds. In: Ayurvedic drugs and their plant sources. 1st edition, New Delhi, CSIR 1992: 119.
3. Kumar D, Ramu P. Effect of methanolic extract of *Benincasa hispida* against histamine and acetyl choline induced bronchospasm in guinea pigs. *Indian J Pharmacol* 2002; 34: 365.
4. Grover JK, Adiga G, Vats V, Rathi SS. Extracts of *Benincasa hispida* prevent development of experimental ulcer. *J Ethnopharmacol* 2001; 78: 159-164.

5. Tandon R, Khanna HD, Dorababu M et al. Oxidative stress and antioxidants status in peptic ulcer and gastric carcinoma. *Indian J Physiol Pharmacol* 2004; 48(1): 115–118.
6. Shay H, Kanmarow SA, Fel SS, Meranze Guenstein M, Simplet H. A simple method for the production of gastric ulceration in the rat. *Gastroenterology* 1945; 5: 43.
7. Stocks J, Dormandy TL. The autooxidation of human red cell lipids induced by hydrogen peroxide. *Br J Hematology* 1971; 20: 95.
8. Beauchamp C, Fridowich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1971; 44(1): 276–287.
9. Roe JH, Keuther CA. *JBC* 1943; 147: 399.
10. Lowry OH, Rosenbrough NS, Fair AL, Randall RJ. Protein measurement with Folin phenol. *JBC* 1932; 193: 265–275.
11. Santra A, Chaudhury A, Chaudhury S et al. Oxidative stress in gastric mucosa in *Helicobacter pylori* infection. *Ind J Gastroenterol* 2000; 19: 21–23.
12. Choi MA, Kim BS, Yu R. Serum antioxidative vitamin levels and lipid peroxidation in gastric carcinoma patients. *Cancer Lett* 1999; 136: 89–93.
13. Joseph RM, Varela V, Kanj VK et al. Protective effects of zinc in indomethacin induced gastric mucosal injury: evidence for a dual mechanism involving lipid peroxidation and nitric oxide. *Aliment Pharmacol Ther* 1999; 13: 203–208.
14. Smith GS, McCreer DW, Cross JM. et al. Gastric injury induced by ethanol and ischemia reperfusion in rat. Differing roles for lipid peroxidation and oxygen radicals. *Dig Dis Sci* 1996; 41: 1157–1164.
15. Yelken B, Dorman T, Erkasap S et al. Clonidine pretreatment inhibits stress induced gastric ulcer in rats. *Anesth Analg* 1999; 89: 159–162.
16. Mandal S, Das DN, De K et al. *Ocimum sanctum* Linn. – A study on gastric ulceration in rats. *Ind J Physiol Pharmacol* 1993; 37(1): 1991–1992.
17. Majumder B, Roychoudhuri Ray A, Bandhyopadhyay SK. Potent antiulcerogenic activity of ethanol extract of leaf of *Piper betle* Linn. by antioxidative mechanism. *Ind J Biochem* 2002; 17(1): 49–57.
18. Yahya MA, Rafatullah S, Ageel AM et al. Gastrocytoprotective effect of ginger *Zingiber officinale* Rose in albino rats. *Am J Clin Biochem* 1989; 17: 51–56.
19. Ray A, Bandhyopadhyay SK. Studies on Indian traditional medicine, *Picrorhiza kurrooa* (Katuki) and peptic ulcer. *Bombay Hosp J* 1998; 40(3): 374–381.
20. Nadkarni KM. *Indian Materia Medica*, Popular Prakashan, Bombay 1976; 480.
21. Yoshizumi S, Murakami T, Kadoya M, Matsuda H, Yamahara J, Yoshikawa M. Medicinal food stuffs. XI. Histamine release inhibitors from wax gourd, fruits of *Benincasa hispida*. *Cogn. Yakaugaku Zasshi* 1998; 118: 188–192.
22. Grassman J. Terpenoids as plant antioxidants. *Vitam Horm* 2005; 72: 505–535.
23. Young-Chan Kim, Mira Jun, Woo-Sik Jeong, Shin-Kyo Chung. Antioxidant properties of Flavone C – glycosides from *Atractylodes japonica* leaves in human low density lipoprotein oxidation. 2005; 70(9): S575–S580.
24. Wagner KH, Elmadfa I. Biological relevance of terpenoids. Overview focusing on mono-, di- and tetraterpenes. *Ann Nutr Metab* 2003; 47(3–4): 95–106.