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AIM AND SCOPES

Cell Membranes and Free Radical Research is a print and online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+ - K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

C- Interaction Between Oxidative Stress and Ion Channels

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

D- Gene and Oxidative Stress (Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics
Biochemistry
Biology
Biomedical Engineering
Pharmacology
Physiology
Genetics
Cardiology
Neurology
Oncology
Psychiatry
Neuroscience

Keywords

Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

Multivitamin-mineral supplement is more efficacious than vitamins C+E in the prevention of chronic unpredictable stress-induced oxidative damage in mice

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List of abbreviations

CAT; catalase
CUS; chronic unpredictable stress
GOT; glutamate oxaloacetate transaminase
GPT; glutamate pyruvate transaminase
GR; glutathione reductase
GSH; reduced glutathione
GST; glutathione S-transferase
LPO; lipid peroxidation
MDA; malondialdehyde
MM; multivitamin-mineral
ROS; reactive oxygen species
SOD; superoxide dismutase
GSH-Px;Glutathione peroxidase

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Abstract

Stress triggers a physiological response by increasing the metabolic rate which translates into oxidative stress, resulting in the etiopathogenesis of many diseases. Several micronutrients like antioxidant vitamins and minerals can modulate the state of oxidative stress. This study tests whether an MM (consisting of functionally diverse dietary antioxidants) compares favourably with a combination of vitamins E and C, in providing increased anti-oxidative protection against chronic unpredictable stress (CUS) induced oxidative damage in mice. Thirty-two Swiss albino mice were randomized to one of the following groups: control+vehicle, CUS+ vehicle, CUS+ MM, and CUS+ vitamins (C+E). CUS was applied for 4 weeks and MM and vitamins (C+E) were administered orally for the same period. CUS led to a negative impact on all the biochemical parameters analyzed in circulation, liver and kidney with elevation of malondialdehyde and reduction of glutathione levels. The activities of superoxide dismutase, catalase, glutathione S-transferase, and glutathione reductase were decreased by CUS, with an elevation of liver marker enzymes, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) in the circulation and liver. Supplementation with MM and vitamins (C+E) restored the disturbed antioxidant status in the stress exposed mice. However, MM was found more effective than vitamins (C+E) in reinstating the altered parameters. The results of the study suggest that the cumulative action of diverse vitamins and minerals in an MM exert greater antioxidative effect than vitamins (C+E) in combating the CUS induced oxidative stress, thus supplementation of MM alone can be an effective measure to combat stress induced oxidative derangements both under normal and pathological conditions.

Keywords

Chronic unpredictable stress, multivitamin-mineral, oxidative stress, vitamin C, vitamin E

Introduction

Stress is a threatening event which elicits various physiological and behavioral responses in an individual. It induces an increase in energy metabolism and promotes oxidative stress which is involved in the etiopathogenesis of many diseases (Zafir and Banu, 2009). In order to mimic chronic stressful experiences faced by human beings in day today life, chronic unpredictable stress (CUS) paradigm (with varied type and timing of stressors) has been widely used in mice (Oritz et al., 1996). Several micronutrients like antioxidant vitamins and minerals can modulate the state of oxidative stress (Richards et al., 2008). Supplements like antioxidant vitamins (C+E) have shown protective effects in human subjects under oxidative stress conditions like extreme exercise (Mastaloudis et al., 2004) and cigarette smoking (Bruno et al., 2006). However in another study, daily ingestion of vitamins E and C were found to abrogate the beneficial effects of physical exercise in humans with type II diabetes (Ristow et al., 2009).

Other kinds of supplements are multivitamin-minerals (MM) which represent a major source of micronutrient intake and are preferred by many individuals for prophylactic purposes (Huang et al., 2007). Antioxidant nutrients such as beta-carotene, vitamin C, vitamin E, selenium and zinc have shown a potential protective effect on the risk of cancer and cardiovascular diseases (Sies et al., 1992). A variety of these supplements have shown a 'modest' extension of mice lifespan (Aksenov et al., 2010). They prevent ageing and neural degeneration by delaying mitochondrial oxidative decay (Ames, 2004). However, efficacy trials evaluating the roles of MM in dissipating stress mediated oxidative damage have not yet been studied.

The objective of the present study was to compare the efficacy of an MM supplement (consisting of multiple dietary nutrients) over antioxidant vitamins in combating the CUS induced oxidative imbalances in the circulation and vital organs of Swiss albino mice. Malondialdehyde (MDA), a marker of lipid peroxidation; antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT); the non-enzymatic such as antioxidant glutathione (GSH); as well as the GSH-metabolizing antioxidant enzymes, glutathione-S-transferase (GST) and glutathione reductase (GR) were also measured in plasma and liver tissue measured. To test the status of liver function, the levels of glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) were estimated in the circulation and liver tissue.

Materials and method

Chemicals

Multivitamin-mineral supplement (Galoxy 490 mg) was purchased from Roots Life Sciences Pvt. Ltd. India; vitamin E (Evion 200 mg) was obtained from Merck, India. Vitamin C (Celin 500mg) was purchased from GlaxoSmithKline Pharmaceuticals Limited, India. NADPH; oxidized and reduced glutathione; 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent); 1-chloro-2, 4-dinitrobenzene (CDNB) and Pyrogallol were acquired from SRL, India. All other chemicals were of analytical grade.

Experimental design

Thirty two healthy male Swiss albino mice each weighing approximately 40g and 5-6 weeks old were acclimatized for 1 week to mice feed (Ashirwad industries, Chandigarh, India) with food and water available ad libitum and alternating light and dark cycles of 12 h. Mice were divided into four groups with 8 mice in each group.

Group I consisted of the non-stressed control mice. Control mice were manipulated everyday for 10 min in the home cage to overcome the nonspecific handling effects.

Group II mice (stress alone) were exposed to 4 weeks of CUS, previously described by Oritz et al (1996). Specific details of the CUS procedure are as follows: for restraint stress, mice were placed individually in body sized wire mesh cages attached to wooden boards, with no movement allowed. Wet bedding was carried out by filling 300 ml tap water in home cage. Forced swim and cold forced swim were accomplished by placing the mice in a cylindrical tank (50 cm height x 20 cm diameter) filled with water to a 20 cm depth at 25 or 4°C, respectively. Crowding was done by placing an iron divider in the cage to provide minimum space for housing. Lastly illumination was attained by placing an illuminated tube light on the

Day	Stress type and schedule
1	1000 h, restraint, 3 h
2	1100 h, wet bedding (25 ºC), 2 h
3	1500 h, forced swim (25 ºC), 30 min
4	1300 h, crowding, 2 h
5	1900 h, lights on, overnight
6	0900 h, cold forced swim (4 ºC), 15 min;
	2200 h, crowding, overnight
7	1000 h, restraint, 2 h
	1900 h, food deprivation, overnight

Table 1 . Weekly chronic unpredictable stress protocol

cages for overnight (Table 1). After each stressor, animals were kept in a recovery room for 1-2 h, following which they were placed in clean cages with fresh bedding and returned to the housing facility.

Group III mice were given oral dose of multivitamin-mineral (200 mg/kg body weight/day) in drinking water (100µl /mouse), followed by chronic unpredictable stressor as in group II. The MM supplement contained: lycopene 6% (2000 µg), α-lipoic acid (50 mg), ß-carotene 10% (10mg), citrus bioflavonoid (50 mg), lutein (250 µg), chromium picolinate (150 µg), selenium dioxide (70 µg), vitamin C (60 mg), vitamin E (15 mg), vitamin D (400 IU), vitamin K (10 µg), vitamin B1 (1.5 mg), vitamin B2 (1.7mg), niacin (20 mg), vitamin B6 (3 mg), folic acid (1.5 mg), vitamin B12 (5 µg), biotin (300 µg), pantothenic acid (10 mg), inositol hexanicotinate (50 mg), calcium (20 mg), phosphorus (48 mg), iodine (150 µg), magnesium oxide (10 mg), manganese sulphate monohydrate (2 mg), molybdenum (75 µg), zinc (15 mg), copper (2 mg), chloride (72 mg), potassium (80 mg), silicon dioxide (2 mg), carbonyl iron (15 mg), boron (150 µg), nickel (5 µg) and vanadyl sulphate (10 µg).

Group IV mice were given oral dose of vitamins (C+E) (100 mg/kg body weight/day, each) followed by chronic unpredictable stressor as in group II. Vitamin C was dissolved in drinking water (100µl /mouse), while vitamin E was given as such. Groups of control and CUS were given vehicle (drinking water, 100 µl/ mouse) orally for the same duration. To avoid product oxidation the supplementations were prepared fresh. Every 7 days, the weight of the animals was measured to adjust the treatment doses.

All the experimental protocols adhered to the guidelines of the Institutional Ethical Committee of the university.

After 4 weeks of CUS paradigm, animals from all the groups were sacrificed by cervical decapitation. Blood, liver and kidney samples were collected for biochemical studies. Liver and kidney samples were rinsed with normal saline and homogenized in 0.1 M phosphate buffer pH 7.4 (10% w/v) followed by centrifugation at 10,000 g (at 4°C for 15 min) to remove cellular debris. Clear supernatant thus obtained, and the plasma separated from blood was utilized for further studies.

Biochemical analysis

Superoxide dismutase (SOD) activity assay

SOD activity was assayed by monitoring the inhibition of auto-oxidation of pyrogallol (0.05 M tris succinate buffer, pH 8.2) at 420 nm. One enzyme unit is defined as the amount of enzyme required to cause 50% inhibition of the rate of pyrogallol auto-oxidation (Marklund and Marklund, 1974).

Glutathione-S-transferase (GST) activity assay

GST activity was assayed in 0.2 M phosphate buffer (pH 6.5) after adding 1mM 1-chloro 2, 4 dinitrobenzene (CDNB) and 1mM GSH in the reaction mixture and following the increase of absorbance at 340 nm due to formation of the CDNB–GSH conjugate. One unit of enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 µM product per min under the specific assay conditions (Habig et al., 1974). Enzyme activity was expressed as units per mg of protein (molar extinction coefficient=9.6×10^3 M/cm).

Catalase (CAT) activity assay

CAT activity was measured in 0.05 M phosphate buffer (pH 7.0) by following the decrease in absorbance at 240 nm due to decomposition of 30 mM hydrogen peroxide (H_2O_2). One enzyme unit is defined as the amount of enzyme decomposing 1 µM H_2O_2 per minute at 25°C (Claiborne, 1985).

Glutathione reductase (GR) activity assay

GR activity was assayed by monitoring the oxidation of 0.1 mM NADPH as a decrease in absorbance at 340 nm due to NADPH-dependent reduction of 1.0 mM oxidized glutathione disulphide to glutathione by the catalytic action of GR (0.1 M phosphate buffer, pH 7.6). One unit of enzyme activity is defined as the amount of enzyme catalyzing the 1 µM NADPH per min under assay conditions (Carlberg and Mannervik, 1975). Enzyme activity was calculated using a molar extinction coefficient of 6.22×10^{-3} M/cm and expressed as units per mg of protein.

Total Reduced glutathione (GSH)

Levels of GSH were determined in circulation, liver and kidney. Homogenates using the classical thiol reagent DTNB (0.1 M phosphate buffer, pH 7.4). The yellow colour developed by the reaction of GSH with DTNB was measured at 412 nm (Jollow et al., 1974).

Lipid Peroxidation (LPO)

Lipid peroxidation was assessed by determining MDA (a thiobarbituric acid reactive species: TBARS) spectrophotometrically following the thiobarbituric acid (TBA) test for the formation of TBARS during an acid-heating reaction (Beuge and Aust, 1978). The pink chromogen formed by MDA–TBA complex was detected

at 535 nm and quantified using an extinction coefficient of 1.56×10^5 M/ cm.

Glutamate Oxaloacetate Transaminase (GOT)/Aspartate transaminase (AST) and Glutamate Pyruvate Transaminase (GPT)/ Alanine transaminase (ALT)

Commercial Kits (Span Diagnostics Ltd, India) were used for the measurement of the transaminases (GOT and GPT) in the liver and plasma.

Protein estimation

Protein content was estimated using bovine serum albumin as standard (Lowry et al., 1951).

Statistical analysis

Data was expressed as group mean ± SEM of eight values and analyzed by one-way ANOVA for differences among controls and treatment groups. P values less than 0.05 were considered statistically significant.

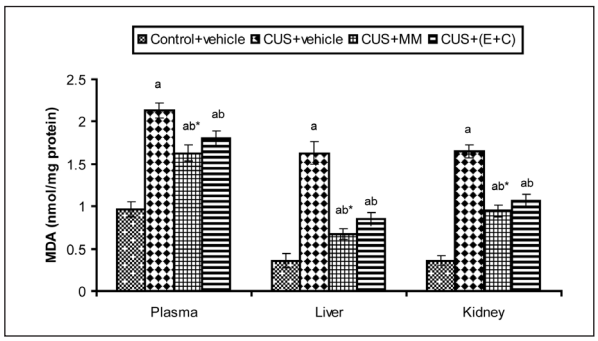

Figure 1. Levels of MDA in circulation, liver and kidney on CUS +vehicle, CUS+ MM; and CUS+ vitamins (C+E) treatments data represent mean ± their SEM of 8 mice. *a, b, P<0.001, a is as compared to controls and b as compared to stressed mice. *P<* 0.05, CUS+MM as compared to CUS+ (C+E) group.

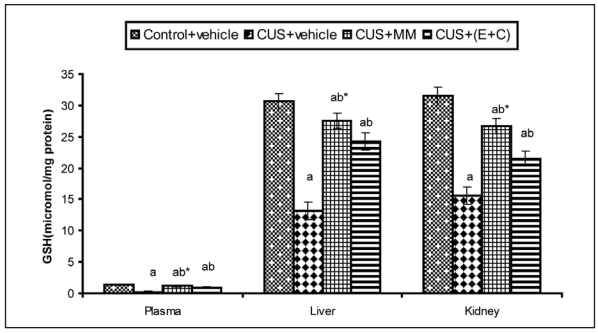

Figure 2. Alterations in the levels of glutathione (GSH) in circulation, liver and kidney by CUS +vehicle, CUS+ MM and CUS+ vitamins (C+E) treatments. Data represent mean ± of 8 mice. *a, b, P*<0.001, a is as compared to controls and b as compared to stressed mice **P<* 0.05, CUS+MM versus to CUS+ (C+E) group.

Results

Four weeks of CUS led to a significant increase of MDA (P<0.001) as compared to controls in the plasma, liver and kidney (Figure 1) tissues of mice. However, simultaneous treatment with MM and vitamins (C+E) significantly lowered (P<0.001) the MDA values when compared with the CUS group. However, decline in MDA levels in the CUS +MM group was significantly more (P<0.05) when compared with the CUS +vitamins (C+E) group.

GSH levels decreased significantly (P<0.001) on stress exposure in the CUS group. Administration of MM and vitamins (C+E) caused a significant (P<0.001) reversion of GSH levels towards their control values in plasma, liver and kidney tissues (Figure 2). GSH levels in the CUS+MM group was significantly higher (P<0.05) when compared to CUS +vitamins (C+E) group.

Antioxidant enzyme activities in plasma, liver and kidney tissues of the treated and control groups are shown in Tables 2, 3 and 4 respectively. Four weeks of stress exposure led to a significant (P<0.001) decline in the circulatory activities of SOD, CAT and GST. In liver and kidney tissues too, the activities of SOD, CAT, GST and GR were significantly reduced (P<0.001) on CUS exposure. MM and vitamins (C+E) treatment to stressed mice significantly (P<0.001) upturned the enzyme activities towards their respective control values. However, recovery in the CUS+ MM group was higher (P<0.05) than in the CUS+ vitamins (C+E) group.

Levels of liver marker enzymes GOT and GPT in CUS group showed significantly increased levels in both plasma (Table 2) and liver (Table 3). However, administration of MM and vitamins (C+E) declined these parameters towards normal levels. Recovery in CUS +MM group was higher (P<0.05) than CUS +vitamins (C+E)

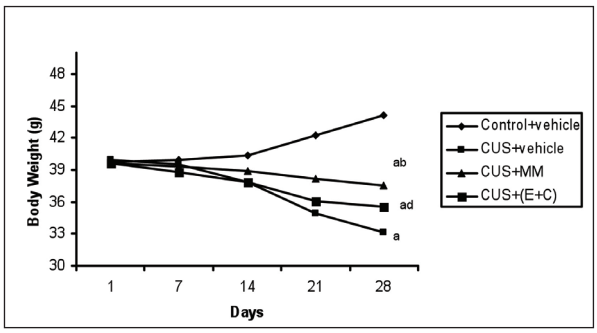

Figure 3. Variations in the body weights of mice in CUS +vehicle, CUS+ MM and CUS+ vitamins (C+E) treatments. Data represent mean ± SEM of 8 mice. *a, b, P*<0.001 and *d, P*<0.05. a is versus controls and *b, d* versus stressed mice.

group, thus proving greater effectivity of MM supplement over vitamins (C+E).

CUS caused a significant (P<0.001) decline in body weights of mice (Figure 3). Treatment of stressed mice with MM and vitamins (C+E) recovered their body weights significantly (P<0.05) when compared to the CUS group. However, attainment of the body weights in CUS +MM and CUS +vitamins (C+E) groups were not significantly different when compared with each other.

In all the cases, MM proved more restitutive than vitamins (C+E) in restoring the antioxidant levels in circulation and tissues of the stressed mice.

Discussion

We observed that 4 weeks of CUS treatment caused a significant decline in the levels of GSH, SOD, CAT, GST and GR with simultaneous increase of MDA levels in the liver, kidney and circulation of mice. Supplementation with MM and vitamins (C+E) to stress exposed mice restored the disturbed antioxidant status, with MM showing a better effect than vitamins (C+E). In the present study, CUS paradigm (with varied type and timing of stressors) was used, that simulates the chronic stressful experiences to which human beings are often exposed in their daily lives (Oritz et al., 1996). CUS led to a deleterious effect on the antioxidant status as well as on the levels

Groups	SGPT	SGOT	SOD	CAT	GST
	(U/ml)		(U/mg protein)		
Control+vehicle	20.56±0.73	13.27±0.49	3.74±0.04	1.26±0.01	1.53±0.02
CUS+vehicle	48.35±0.99[a]	35.98±0.70[a]	0.94±0.01[a]	0.77±0.01[a]	0.43±0.01[a]
CUS+MM	35.74±0.64[ab*]	27.05 ±0.37[ab*]	2.38±0.04[ab*]	1.13 ±0.03[cb*]	1.24±0.01[ab*]
CUS+(C+E)	39.53±0.45[ab]	29.40±1.18[ab]	2.04±0.02[ab]	1.07±0.01[ab]	0.98±0.01[ab]

Table 2 . Data represent mean with their SEM of 8 mice

[a] *P*<0.001 and [c]*P*<0.01, versus controls

[b] *P*<0.001, versus stressed mice

**P*< 0.05, CUS+MM versus CUS+ (C+E) group

Groups	GPT	GOT	SOD	CAT	GST	GR
	(U/ml)		(U/mg protein)			(U/mg protein x 10^{-3})
Control+vehicle	78.36±0.71	74.49±0.65	165.52±0.24	98.71 ±0.39	95.64±0.33	1.99±0.07
CUS+vehicle	96.96±0.41[a]	89.66±0.69[a]	88.00±0.39[a]	70.52±0.44[a]	90.52 ±0.32[ab*]	0.96±0.07[a]
CUS+MM	87.29±0.41[ab*]	81.35± 0.47[ab*]	115.63±0.44[ab*]	90.52 ±0.32[ab*]	92.48±0.36[ab*]	1.67±0.05[ab*]
CUS+(C+E)	90.66±0.55[ab]	82.87±0.48[ad]	106.41±0.44[ab]	85.46±0.24[ab]	88.38±0.28[ab]	1.42±0.05[ab]

Table 3 . Data represent mean with their SEM of 8 mice

P<0.001, versus controls

[b] *P*<0.001 and [d]*P*<0.01, versus stressed mice

**P*< 0.05, CUS+MM versus CUS+ (C+E) group.

Groups	SOD	CAT	GST	GR
	(U/mg protein)			(U/mg protein x 10^{-4})
Control+vehicle	165.37±0.29	115.7 ±0.31	37.62±0.41	5.61±0.05
CUS+vehicle	46.49±0.31[a]	62.46±0.26[a]	17.36±0.47[a]	0.50±0.08[a]
CUS+MM	127.61±0.29[ab*]	101.56 ±0.27[ab*]	29.92±0.33[ab*]	3.98±0.04[ab*]
CUS+(C+E)	111.55±0.28[ab]	96.46±0.29[ab]	25.23±0.49[ab]	1.78±0.02[ab]

Table 4 . Data represent mean with their SEM of 8 mice

[a]*P*<0.001, versus controls

[b]*P*<0.001, versus stressed mice

**P*< 0.05, CUS+MM versus CUS+ (C+E) group.

of biochemical markers in circulation and the tissues. Antioxidant enzymes SOD and CAT are frequently used as markers of oxidative stress and they are important in the preservation of homeostasis for normal cell function. CUS caused a significant diminution in the activities of SOD and CAT in plasma, liver and kidney tissues. Treatment with MM and vitamins (C+E)increased the activities of these enzymes to near normal levels, but MM proved more effective than vitamins (C+E). MM contained along with the main antioxidant vitamins such as vitamin E, vitamin C and β-carotene, some minerals as Cu, Zn, Mn and Fe, which act as co-factors for the antioxidant enzymes superoxide dismutase and catalase. Also, Cu is necessary to adequate Fe utilization, which is an important component of catalase. Therefore, supplementation with these micronutrients strengthened the enzymatic antioxidant system.

GST, a phase II enzyme, is thought to play a physiologic role in initiating the detoxification of the products of oxidative stress. It can directly scavenge ROS to reestablish the homeostatic redox tone of normal cells (Lee and Surh, 2005). On CUS exposure activity levels of GST, GR and GSH were found to decrease significantly, indicating a hampered glutathione metabolism and a weakened antioxidant defense system. The decrease in GR may be due to the ill effects of free radicals on the enzyme, which in turn caused a decrease in the level of reduced glutathione. However, both MM and vitamins (C+E) increased their activities, with a pronounced effect by MM. Greater increase in the activities of phase II detoxification agents (GST, GR and reduced GSH) in the circulation and tissues of CUS+ MM treated mice implies that MM was more effective in the detoxification of endogenous compounds such as peroxidised lipids than antioxidant vitamins alone. Further, the effectiveness of MM over combined vitamins (C+E) is depicted by decrease/restoration in the levels of MDA, a good indicator of oxidative injury, which was found elevated in the circulation and tissues after 4 weeks of CUS exposure. The increase in LPO could be due to a significant rise in the generation of H_2O_2 along with depleted GSH during stress exposure. A similar enhancement of LPO on CUS treatment has been reported earlier in mice liver and kidney (Zhang et al., 2009; Nayantara et al., 2009). The restoration of MDA levels may be due to modulation of *in vivo* antioxidant system which might have decomposed the peroxides and thus offering a protection against lipid peroxidation. The more effective antioxidant action of MM may be due, at least in part, to the induction of higher levels of reduced glutathione, other antioxidants and GST a phase II detoxification enzyme.

Levels of SGOT and SGPT, marker enzymes for hepatic functional status, increased in plasma and liver on stress exposure, which demonstrates liver malfunction due to cellular necrosis in hepatocytes and increased membrane permeability. Supplementation with MM and vitamins (C+E) lowered their levels. This can be due to the ability of these agents to inhibit stress induced activation of Hypothalamic-Pituitary-Adrenal (HPA)-axis and sympathetic system. However, MM more effectively normalized the levels as compared to vitamins (C+E) which can be attributed to their higher antioxidative prowess. By scavenging the free radicals, MM prevented the oxidative damage to macromolecules, such as proteins and lipids, which would lead to the loss of their biological properties and eventually to cell death.

Body weights of mice were significantly decreased on stress exposure. According to Martí et al. (1994) the reduction on body weight gain induced by stress is mediated, at least in part, by anorexia. Supplementation with MM and vitamins (C+E) prevented the loss in body weight.

As a novel result of the current study, the MM showed significantly higher antioxidant potential than vitamins (C+E) in normalizing the activities/levels of the imbalanced biochemical parameters. A combination of functionally diverse dietary antioxidants and the synergistic effects among them may account for the enhanced protection in circulation and tissues compared to the results obtained by supplementing with vitamins (C+E) alone. The exogenous nutrients provided by MM interact with the endogenous antioxidants and help in restoring the altered oxidant status. Multiple nutrients in a supplement would provide complementary antioxygenic functions in both lipophilic and aqueous media. They comprise both preventive and chain-breaking antioxidants. Carotenoids and flavonoids are excellent preventive antioxidants, due to their ability to quench singlet oxygen (Ratty et al., 1988). Whereas vitamins E and C are potent chain-breaking antioxidants, due to their large rate constants for the reaction with peroxyl radicals (Chert and Tappel, 1996).

Previous studies in our lab have demonstrated the modulation of CUS induced oxidative damage in brain and heart of mice by MM and vitamins (C+E). It was found that the MM more effectively upturned the decreased activities of SOD, CAT, GST and GR and normalized the levels of GSH and MDA (Hasan et al.,

2010). Trace elements such as Cu and Se are essential components of the antioxidants ceruloplasmin and glutathione peroxidase respectively (Mckee and Frieden, 1971; Levander, 1974). Vitamin B6, Fe, Cu, Zn, riboflavin, biotin, lipoic acid and pantothenic acid participate in heme metabolism, which is important in regulating the release of reactive oxidants (Atamna and Frey, 2004; Atamna, 2004). Magnesium ions are a required cofactor in mitochondrial electron transport chain complex subunits, methylenetetrahydrofolate dehydrogenase 2, and pyruvate dehydrogenase phosphatase (Bogucka and Wojtczak, 1976). Studies on magnesium deficient cultured human cells and animals show evidence of decreased antioxidant defenses (Dickens, 1992; Malpuech-Brugere et al., 1999).

In conclusion, the results of the present study supported the concept of involvement of free radicals as a physiological response to stress and the prophylactic role of MM through an antioxidant mechanism. Hence MM can be proposed as a complete supplement in optimizing human health and is likely to have great health benefits particularly to those with imbalanced nutrition like poor, young, obese and elderly people. Further, an MM supplementation can prove beneficial to nutrition compromised patients suffering from severe diseases like cancer, cardiovascular diseases, cataract and other pathological disorders, as they face high level of psychological and physical stress which can sabotage their therapy.

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