

The Chemo-Protective Effect of Turmeric, Chili, Cloves and Cardamom on Correcting Iron Overload-Induced Liver Injury, Oxidative Stress and Serum Lipid Profile in Rat Models.

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Abstract: Aim of the work: Assessing the chemoprotective effect of turmeric, clove, chili and cardamom on correcting iron overload -inducing liver injury ,oxidative stress and serum lipid profile in rat models.

Methods: Design of the Study: Thirty six male wistar rats weighing 145.81 ± 12.10 g (mean \pm S.D) were divided into 6 groups. The first group served as a control and consumed a standard diet according to (AIN – 93). The other 5 groups were injected intraperitoneally with a single dose 30 mg Fe / kg body weight. One group was kept without further treatment and served as a positive control. Groups 3, 4, 5 and 6 consumed diets to which finely ground 2 % turmeric, clove, and chili and cardamom flour were incorporated , respectively. The feeding trial continued for five weeks. At the end of the experiments, the animals were sacrificed, blood samples were collected, and the liver was separated and saved frozen for subsequent biochemical analysis. Laboratory investigations Consisted of liver function test [ALT, AST, ALP], complete lipid profile, measurement of lipid peroxidation indices and the antioxidative catalase enzyme activity. In addition liver iron was determined to test the magnitude of liver toxicity and the antioxidant potential of the supplements. Results: The iron overload was associated with significant increases in the activities of the liver enzymes AST, ALT, ALP ($P < 0.05$) compared with the respective mean control values. All parameters of lipid profile (i.e., serum total cholesterol, triacylglycerol, LDL-cholesterol, phospholipids), total bilirubin and MDA showed significant increase. On the other hand, the mean HDL – cholesterol and the activity of serum catalase were lower than the respective mean values of the control. Liver iron deposition also increased significantly after the iron overload. The incorporation of the turmeric, clove, chili, or cardamom in the diet at 2 % significantly restored the enzyme activities of the liver AST, ALT, ALP to normal level. The mean values of lipid profile, the MDA and serum total bilirubin were also reduced. The liver iron deposition was reduced with significant increase in the activity of mean serum catalase and HDL-cholesterol compared with the respective mean values obtained with the positive control group overloaded with iron. Conclusion: The observed improvement in the liver functions suggests that the chemo-protective effect of the turmeric, clove, chili and cardamom is attributed to chelation with iron followed by excretion of the complex. This result may find application among populations at risk of iron overload; either acquired or inherited. [Journal of American Science 2010;6(10):702-712]. (ISSN: 1545-1003).

Key words: Iron overload, liver injury, oxidative stress, turmeric, clove, chili and cardamom.

1. Introduction:

Iron is an essential cofactor for important biological activities and biochemical reactions, including the transport of oxygen via red blood cells and its reduction to water during respiration. While iron's bioavailability is generally limited, pathological accumulation of the metal within tissues aggravates the generation of reactive oxygen species (ROS) and elicits toxic effects, which are mainly related to oxidative stress (Galaris and Pantopoulos .,2008). As a redox-active transition metal, iron generates reactive oxygen species (ROS) via the Fenton and Haber–Weiss reactions. ROS react directly with proteins, lipids and nucleic acids and induce oxidative stress by depleting cellular stores of antioxidants. ROS also influence multiple cell

signaling pathways important to cell survival, proliferation and death (Valko et al.,2005).

Iron overload can cause liver toxicity and increase the risk of liver failure or hepatocellular carcinoma in humans (Messner et al., 2009)

Iron overload syndromes are classified as genetic (hereditary hemochromatosis) or secondary (most commonly in patients who require long-term blood transfusions, as in severe anemias and thalassemia). In addition, there are many diseases that show mild iron deposition or dysregulation of body iron distribution. Such conditions include chronic hepatitis C, alcoholic liver disease and non-alcoholic teatohepatitis (Britton et al., 1994; Kohgo et al., 2008).

In addition, it has been demonstrated that FeNTA–induced oxidative stress could lead to

hepatocyte apoptosis (Yajun et al., 2005), DNA damage and liver necrosis in rats (Matos et al., 2001). Several phytochemicals, derived from vegetables, fruits, herbs and spices, have demonstrated excellent chemopreventive properties against carcinogenesis by regulating the redox status of the cells during oxidative stress. (Acharya et al., 2010).

Galaris and Pantopoulos (2008) reported that turmeric (*Curcuma longa* rhizomes), commonly used as a spice, is well documented for its medicinal properties in Indian and Chinese systems of medicine. It has been widely used for the treatment of several types of diseases (Maheshwari et al., 2006). Turmeric consumption may reduce the risk of some form of cancers and render other protective biological effects. (Balcerek et al., 2005). These biological effects of turmeric have been attributed to its constituent curcumin that has been widely studied for its antioxidant, anti-inflammatory, anti-angiogenic, wound-healing and anti-cancer effects (Duvoix et al., 2005). Also it has potential therapeutic effects against neurodegenerative, cardiovascular, pulmonary, metabolic and autoimmune diseases (Aggarwal and Harikumar, 2009). In addition, curcumin exerted hepatoprotective effects in various animal models of liver injury such as carbon tetrachloride (Park et al., 2000; Fu et al., 2008), endotoxin (Kaur et al., 2006) and thioacetamide (Shapiro et al., 2006).

Red chili (RC) (*Capsicum frutescens* L.) is widely used as a spice for flavoring foods, particularly in South-East Asian and Latin-American countries. The major active ingredients of RC are pungent capsaicinoids (capsaicin, dihydrocapsaicin), antioxidant vitamins (ascorbic acid, vitamin E), carotenoids (β -carotene, β -cryptoxanthine) and several organic acids and minerals (Antonious et al., 2006; Conforti et al., 2007). Oboh et al., 2006 reported that hot peppers prevent Fe²⁺-induced lipid peroxidation,

Jirovetz et al. (2006) indicated that clove (*Eugenia Caryophyllata*) and cardamom (*Amomum Subulatum*) are among the widely used spices. Clove is used to help digestion, prevent vomiting in pregnancy and has inhibitory effect on histamine production. whereas, Kikuzaki et al., 2001 showed that cardamom has antispasmodic action. Also clove was classified (Sharma et al., 2001) as a source for power antioxidant activity, whereas, cardamom had a medium level antioxidant. In addition to its effectiveness in reducing LDL susceptibility to oxidation (Nair et al., 1998).

The present study was designed to determine and to compare the chemo-protective effect of turmeric (*curcuma longa*), Red Chili (*Capsicum frutescens* L.), Cloves (*Syzygium aromaticum*),

Cardamom (disambiguation) on correcting iron overload-induced liver injury, oxidative stress and serum lipid profile in rat models.

2. Materials and Methods

Materials:

Chemicals:

All chemicals were fine grade, chemicals purchased from local distributor (Sigma chemical) Cairo, Egypt.

Spices including turmeric (*curcuma longa*), Red Chili (*Capsicum frutescens* L.), Cloves (*Syzygium aromaticum*), Cardamom (disambiguation) powder were purchased from a local market, Cairo, Egypt and was mixed with basal diet.

The basal standard diet was prepared in accordance with AIN-93 formulation (Revees et al., 1993).

Animals

In the present study 36 male rats of wistar strain weighing (145.81 \pm 12.10g) obtained from Institute of Ophthalmology (Cairo, Egypt) were used in this study. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in stainless steel cages one per cage at temperature 22 \pm 3 °C and relative humidity 30-70 %. The animal diet was given ad libitum. Animals were acclimatized for one week prior to experiment.

Thirty six rats were divided into 6 groups each of 6 rats.

Group 1(G1): served as normal control and received standard diet.

Each animal in the Fe-loaded group (group 2) and the other four treated groups (group 3, 4, 5 and 6) received a single I.P injection of an iron dextran complex (Sigma Chemical Co., St. Louis, MO, USA) at a dose of 30 mg elemental iron/kg body weight.

Group 2(G2): served as control positive and received standard diet

Group 3(G3): received standard diet + 2% turmeric.

Group 4(G4): received standard diet + 2% clove.

Group 5(G5): received standard diet + 2% red pepper.

Group 6(G6): received standard diet + 2% cardamom.

Treatments started one week before and concurrently after iron administration.

The experiment lasted for 5 weeks.

Assays:

At the end of experimental period, all rats were fasted overnight and then anesthetized by ether and sacrificed. Blood was collected and allowed to clot; serum was separated by centrifugation at 3000 rpm for 15 minutes serum was then transferred into

properly labeled sterile vials and stored at -20°C till the performance of Laboratory analysis.

Liver, kidney and spleen were excised, rinsed in chilled saline solution and then blotted on filter paper, weighed separately to calculate the relative weight.

The relative weight of organ = $\frac{\text{absolute weight of organ}}{\text{Final body weight of rat}} \times 100$

Tissue homogenate: Liver was excised, washed with saline and stored at -70°C till estimation of liver iron.

Serum was used for determination of AST and ALT activities according to (Henry et al., 1960), serum ALP according to (King and King, 1954). Serum phospholipids was assayed according to (Connerty et al., 1961) and total bilirubin was determined by the method of (Fevery et al., 1976). Serum total cholesterol was assayed by the method of Richmond, (1973), serum triacylglycerol according to Fossati and Prencipe, (1982), serum HDL by the method of Steele et al., (1976) while serum LDL-cholesterol by the use of the equation of Friedewald et al., (1972). MDA was measured as an indication of lipid peroxidation using the colorimetric method described by Draper and Hadly, (1990). Serum catalase was assayed according to Vanizor et al., (2003). Liver samples were digested using advanced microwave digestion system. Liver iron deposition was. Liver iron was measured using ICP Spectrometer (ICPA 6000 SERIES; thermo scientific) according to Imre et al., (2005).

Statistical analysis:

Statistical analysis: were performed using SPSS for Windows 10.0 (SPSS Inc, Chicago, IL, USA). Data were expressed as mean \pm S.D. One way analysis of variance (ANOVA) at ($p < 0.05$) was used to compare mean values of continuous variable in cases and control.

3. Results

The present findings indicated that there is no significant difference in weight gain and food intake/day (Table 1) between all treatment group. However, there is a significant increase ($p < 0.05$) in relative weights (g/100g body wt) of liver and kidney in iron overloaded- rats compared to control group (group 1). Relative weight of liver was significantly

decreased ($p < 0.05$) with treatment of either 2% turmeric, clove, chili, or cardamom (Table 1)

As shown in table 2 there is a significant increase ($p < 0.05$) in the activities of AST, ALT, ALP, total bilirubin, in group 2 as compared to control group (group 1). Supplementation with either 2% turmeric, clove, chili, or cardamom significantly decreased ($p < 0.05$) serum AST, ALT, ALP, total bilirubin compared to iron overloaded group with the lowest value in group 4 fed 2% clove.

Iron overload caused many adverse effects reflected on the significant increase ($p < 0.05$) of serum total cholesterol, triacylglycerol, LDL-C, VLDL-C, phospholipids in group 2 as compared to all five groups. Supplementation with either 2% turmeric, clove, chili, or cardamom significantly decreased ($p < 0.05$) serum total cholesterol, triacylglycerol, LDL-C, VLDL-C, phospholipids compared to iron overloaded group with the lowest values in group 3 fed 2% turmeric except for triacylglycerol where the lowest value was for group 6 fed 2% Cardamom (Table 3).

On the other hand there is a significant decrease ($p < 0.05$) in serum HDL-cholesterol in group 2 compared to the control group (group 1). Supplementation with either 2% turmeric, clove, chili, or cardamom significantly increased ($p < 0.05$) serum HDL-cholesterol when compared to control positive iron overloaded group with the highest value in group 5 fed 2% chili (Table 3).

There is a significant increase ($p < 0.05$) of serum MDA and liver iron deposition in group 2 as compared to control group (group 1). Supplementation with either 2% turmeric, clove, chili, or cardamom significantly decreased ($p < 0.05$) serum MDA and liver iron deposition compared to iron overloaded group with the lowest value in group 4 fed 2% clove (Table 4).

On the other hand there is a significant decrease ($p < 0.05$) in serum catalase in group 2 compared to the control group (group 1). Supplementation with either 2% turmeric, clove, chili, or cardamom significantly increased ($p < 0.05$) serum catalase when compared to iron overloaded group with the highest value in group 4 fed 2% clove (Table 4).

Table (1): Effect of turmeric, clove, chili, and cardamom on weight gain, food intake/day and relative weights of different organs (liver, kidney & spleen) In Iron overloaded- rats (Mean \pm S.D.).

| Groups | Group (1) Normal control | Group (2) Iron overload | Group (3) Turmeric + Iron overload | Group (4) Clove + Iron overload | Group (5) Chili + Iron overload | Group (6) Cardamom + Iron overload |
|--------------------------------|-----------------------------|------------------------------|---------------------------------------|------------------------------------|------------------------------------|---------------------------------------|
| Weight gain(g) | 57.12 \pm 11.67 | 59.33 \pm 11.11 | 56.50 \pm 8.11 | 50.17 \pm 5.53 | 56.17 \pm 8.2 | 58.33 \pm 5.13 |
| Food intake(g/day) | 16.35 \pm 1.25 | 15.68 \pm .84 | 15.60 \pm 1.78 | 16.10 \pm 1.65 | 15.93 \pm 1.44 | 15.90 \pm 1.47 |
| Relative weight of liver (g%) | 2.34 \pm 0.16 | 2.92 \pm 0.04 ^a | 2.76 \pm 0.16 ^{a,b} | 2.68 \pm 0.21 ^{a,b} | 2.54 \pm 0.11 ^b | 2.60 \pm 0.28 ^b |
| Relative weight of kidney (g%) | 0.49 \pm 0.07 | 0.62 \pm 0.04 ^a | 0.61 \pm 0.03 ^a | 0.56 \pm 0.03 | 0.61 \pm 0.02 ^a | 0.61 \pm 0.02 ^a |
| Relative weight of spleen (g%) | 0.25 \pm 3.08 | 0.242 \pm 1.52 | 0.21 \pm 1.40 | 0.26 \pm 7.38 ^{c,e,f} | 0.19 \pm 2.33 ^{a,d} | 0.21 \pm 2.88 |

Significant difference ($P < 0.05$): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5, (f): to group 6.

Table (2) : Effect of turmeric , clove, chili, and cardamom on serum ALT , AST , ALP and on serum total bilirubin in iron overloaded- rats (Mean \pm S.D.).

| Groups | Group (1) Normal control | Group (2) Iron overload | Group (3) Turmeric + Iron overload | Group (4) Clove + Iron overload | Group (5) Chili + Iron overload | Group (6) Cardamom + Iron overload |
|------------------------|-----------------------------|-------------------------------|---------------------------------------|------------------------------------|------------------------------------|---------------------------------------|
| ALT(UL) | 26.61 \pm 0.59 | 43.94 \pm 2.63 ^A | 27.06 \pm 0.99 ^b | 24.43 \pm 0.51 ^{a,b,c} | 25.81 \pm 0.69 ^b | 25.45 \pm 0.73 ^{b,c} |
| AST(UL) | 31.48 \pm 0.51 | 44.44 \pm 1.47 ^a | 32.35 \pm 1.37 ^b | 30.33 \pm 0.41 ^{a,b,c} | 31.58 \pm 0.46 ^b | 31.48 \pm 0.52 ^{b,d} |
| ALP (UL) | 38.58 \pm 0.13 | 49.40 \pm 1.08 ^A | 39.73 \pm 1.12 ^{a,b} | 38.53 \pm 0.85 ^{b,c} | 38.57 \pm 0.56 ^{b,c} | 38.87 \pm 0.73 ^b |
| Total bilirubin(mg/dl) | 5.42 \pm 0.45 | 9.23 \pm 0.65 ^A | 8.23 \pm 0.12 ^{a,b} | 7.82 \pm 0.17 ^{a,b} | 8.77 \pm 0.37 ^{a,b,c,d} | 8.44 \pm 0.25 ^{a,b,d} |

Significant difference ($P < 0.05$): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5, (f): to group 6.

Table (3) : Effect of turmeric , clove, chili, and cardamom on serum total- cholesterol; triacylglycerol; LDL-C ; HDL-C ;VLDL-C and phospholipids on iron overloaded- rats(Mean \pm S.D.).

| Groups | Group (1) Normal control | Group (2) Iron overload | Group (3) Turmeric + Iron overload | Group (4) Clove + Iron overload | Group (5) Chili + Iron overload | Group (6) Cardamom + Iron overload |
|--|-----------------------------|--------------------------------|---------------------------------------|------------------------------------|--------------------------------------|--|
| Total- cholesterol (TC ,mg/dl) | 96.30 \pm 1.34 | 168.00 \pm 1.49 ^a | 110.15 \pm 3.25 ^{a,b} | 123.62 \pm 1.29 ^{b,c} | 123.37 \pm 1.83 ^{a,b,c,d} | 118.65 \pm 1.17 ^{a,b,c,d,e} |
| Triacylglycerol (mg/dl) | 97.44 \pm 1.12 | 138.80 \pm 1.53 ^a | 110.70 \pm 1.50 ^{a,b} | 115.78 \pm 1.20 ^{a,b,c} | 117.66 \pm 0.86 ^{a,b,c,d} | 108.08 \pm 1.06 ^{a,b,c,d,e} |
| High density lipoprotein cholesterol (mg/dl) | 45.03 \pm 2.16 | 38.23 \pm 1.02 ^a | 44.34 \pm 1.58 ^b | 45.51 \pm 0.92 ^b | 47.38 \pm 0.46 ^{a,b,c,d} | 44.65 \pm 0.82 ^{b,e} |
| Low density lipoprotein cholesterol mg/dl) | 31.76 \pm 1.14 | 102.00 \pm 1.85 ^a | 43.72 \pm 2.47 ^{a,b} | 54.95 \pm 0.94 ^{a,b,c} | 52.45 \pm 1.80 ^{a,b,c} | 54.61 \pm 5.98 ^{a,b,c} |

| | | | | | | |
|--|--------------|---------------------------|------------------------------|-------------------------------|---------------------------------|-----------------------------------|
| Very low density lipoprotein cholesterol (mg/dl) | 19.49 ± 0.22 | 27.77 ± 0.29 ^a | 22.14 ± 0.299 ^{a,b} | 23.16 ± 0.24 ^{a,b,c} | 21.63 ± 0.21 ^{a,b,c,d} | 22.95 ± 2.56 ^{a,b,c,d,e} |
| Phospholipids (mg/dl) | 36.54 ± 0.69 | 86.53 ± 0.82 ^a | 46.88 ± 0.74 ^{a,b} | 49.72 ± 1.16 ^{a,b,c} | 49.21 ± 0.68 ^{a,b,c} | 47.85 ± 0.71 ^{a,b,c,d,e} |

Significant difference ($P < 0.05$): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5, (f): to group 6.

Table (4) : Effect of turmeric , clove, chili, and cardamom on serum MDA and serum catalase as well on liver iron in iron overloaded- rats(Mean ± S.D.).

| Parameters | Group (1) Normal control | Group (2) Iron overload | Group (3) Turmeric + Iron overload | Group (4) Clove + Iron overload | Group (5) Chili + Iron overload | Group (6) Cardamom + Iron overload |
|-----------------------|-----------------------------|----------------------------|---------------------------------------|------------------------------------|------------------------------------|---------------------------------------|
| MDA (nmol/l) | 1.82 ± 0.39 | 3.89 ± 0.49 ^a | 2.22 ± 0.35 ^{b,f} | 1.68 ± 0.37 ^{b,c} | 1.95 ± 0.35 ^b | 2.47 ± 0.38 ^{a,b,d} |
| Catalase (U/L) | 407.87 ± 1.14 | 385.62 ± 0.92 ^a | 401.13 ± 2.19 ^{a,b} | 404.35 ± 2.51 ^{a,b,c} | 300.37 ± 3.04 ^{a,b,d} | 398.77 ± 2.52 ^{a,b,d} |
| Liver iron (mg/100 g) | 20.03 ± 1.47 | 39.86 ± 1.72 ^a | 24.79 ± 1.00 ^{a,b} | 19.48 ± 1.64 ^{a,b} | 22.57 ± 0.78 ^{a,b,c,d} | 28.90 ± 0.36 ^{a,b,d} |

Significant difference ($P < 0.05$): (a) compared to group 1, (b): to group 2, (c): to group 3, (d): to group 4, (e): to group 5, (f): to group 6.

4. Discussion

Significant increase ($p < 0.05$) in relative weights(g/100g body wt) of liver , kidney in iron overloaded- rats is in agreement with those of Whittaker and Chanderbhan ,(2001) who found that there was an enlargement of the liver and heart with increasing Fe dose when comparing the organ weights to body weight.

Relative weight of liver is significantly decreased ($p < 0.05$) with either 2% turmeric, clove, chili, or cardamom

Naik , et al., (2010) showed that the increase in relative weight of liver and heart in CCl₄ induced liver injury and isoproterenol induced cardiac necrosis were reduced by curcumin treatment.

Iron overload caused many adverse effects including a significant increase ($p < 0.05$) in the activities of AST, ALT, ALP, total bilirubin, in group 2 as compared to control group (group1). These results are in accordance with Whittaker and Chanderbhan , (2001) who found that iron overload caused many adverse effects reflected the significant increase of serum ,AST, ALT and ALP and Pulla Reddy and Lokesh , (1996) who found that male wistar rats injected i.p. with 30 mg Fe²⁺/kg body weight show hepatic damage as measured by an increase in lipid peroxides which correlated with

elevated serum enzymes, (ALT), (AST) and lactate dehydrogenase (LDH). In another study by Manjunatha and Srinivasan ., (2006) ,rats injected with iron showed hepatic toxicity as measured by an increase in lipid peroxides and elevated serum enzymes, ALT, AST and LDH. Such increased activities might be attributed to the leakage of these enzymes from the injured liver cells into the blood stream because of the altered liver membrane permeability.

Supplementation with either 2% turmeric , clove, chili, or cardamom significantly decreased ($p < 0.05$) serum AST, ALT, ALP, total bilirubin compared to iron overloaded group with the lowest value in group 4 fed 2% clove .These results are in agreement with Pulla Reddy and Lokesh ,(1996) Who found that oral administration of spice principles, curcumin from turmeric (30 mg/kg body weight) or eugenol from cloves (100 mg/kg body weight), for 10 days lowered the liver and serum lipid peroxide levels, serum ALT, AST and LDH, enhanced by i.p. injection of iron. This study indicates that curcumin or eugenol reduces the iron-induced hepatic damage by lowering lipid peroxidation. EL-Maraghy et al ., (2009) demonstrated a reduction in the severity of iron-induced hepatotoxicity by curcumin through the correction of the altered liver function indices. Reyes-

Gordillo K., et al., (2008) reported that curcumin was effective in preventing and reversing cirrhosis, probably by its ability of reducing transforming growth factor-beta (TGF-beta) expression. Thus curcumin might be an effective antifibrotic and fibrolitic drug in the treatment of chronic hepatic diseases.

Also Fu Y, et al., (2008) demonstrated that curcumin significantly protects the liver from injury by reducing the activities of serum AST, ALT, and ALP, and by improving the histological architecture of the liver.

The effect of curcumin on prevention of acute liver damage can be explained by at least two mechanisms: acting as an antioxidant and by inhibiting NF-kappaB activation and thus production of proinflammatory cytokines. (Reyes-Gordillo K et al., 2007). Naik SR, et al., (2010) showed that curcumin treatment reversed elevated serum marker enzymes, (AST), (ALT) and (ALP), increased lipid peroxidation, decreased glutathione (GSH), glutathione peroxidase (GPx) and superoxide dismutase (SOD) in edematous, granulomatous, liver and heart tissues during inflammation, liver injury and cardiac necrosis, respectively. Abdel-Wahab., (2005) observed that treatment with clove and cardamom effectively decreased liver enzyme levels in the serum. This can be attributed to the presence of antioxidant in clove and cardamom which contain phenolic compounds that can act by scavenging free radicals.

Nagababu et al., (2010) reported that eugenol significantly inhibited the rise in SGOT activity and cell necrosis without protecting the endoplasmic reticulum (ER) damage as assessed by its failure to prevent a decrease in cytochrome p450 and G-6-phosphatase activity.

The significant increase ($p < 0.05$) of serum total cholesterol, triacylglycerol, LDL-cholesterol, VLDL-cholesterol, phospholipids and the significant decrease ($p < 0.05$) in serum HDL-cholesterol in group 2 compared to the control group are in line with similar finding reported by Whittaker and Chanderbhan, (2001) who found that serum cholesterol concentration increased directly with Fe supplementation. Also, Brunet et al., (1999) found a significant increase in cholesterol and triglycerides in iron overloaded rats when compared with control animals. They also found a reduction in 3-hydroxy-3-methylglutaryl-Co A reductase and cholesterol 7 α -hydroxylase, and an enhancement of acyl-Co A-cholesterol acyltransferase activity. They reported that this may have been a result of marked membrane lipid peroxidation that brings about fluidity drop in microsomes of Fe-loaded rats. Dabbagh et al., (1994) also found that Fe overload in male Sprague-

Dawley rats caused a significant increase in plasma cholesterol and moderately increased lipid peroxidation in the liver. The increase in plasma cholesterol was explained by a decrease in antioxidant levels in plasma and liver. Sylvain et al., (2003) found iron-overload rats showed a significant increase in triglycerides, free cholesterol, cholesterol ester.

Whittaker and Chanderbhan, (2001) reported that lipid peroxidation and cytotoxicity are important in altering lipid metabolism, possibly by their effect on lipogenesis-related genes (Foretz et al. 1999) and key enzymes for cholesterol homeostasis (Brunet et al. 1999). Damage to cellular lipids may result in structural alterations, such as membrane fluidity and fragility, and in functional alterations. Oxidative changes in lipoproteins may result in altered lipoprotein-receptor interaction in extrahepatic target cells, leading to changes in serum lipid profiles. The oxidative change in the liver may result in alterations in sterol synthesis, leading to increased serum cholesterol levels with concurrent increases in serum phospholipids and changes in the ratios of their saturated to unsaturated fatty acids.

The significant cholesterol lowering effect of turmeric and chili disagree to some extent with finding of Manjunatha and Srinivasan, (2007) who reported that curcumin, capsaicin, and their combination produced only a slight decrease in serum total cholesterol in animals. However, they reported that serum alpha-tocopherol content was increased by dietary curcumin, capsaicin, and their combination in high-fat-fed rats. Serum total thiol content in high-fat-fed animals and serum ascorbic acid in normal animals were elevated by feeding a combination of curcumin and capsaicin, whereas, lipid peroxide level was reduced by dietary curcumin and combination of curcumin and capsaicin in high-fat-fed animals. Serum glutathione peroxidase and glutathione transferase in high-fat-fed rats were generally higher as a result of dietary curcumin, capsaicin, and the combination of curcumin and capsaicin.

Manjunatha and Srinivasan (2006) demonstrated that, individually, both dietary curcumin and capsaicin significantly inhibited the in vivo iron-induced LDL oxidation, as well as copper-induced oxidation of LDL in vitro. The protective effect of the combination of curcumin and capsaicin on LDL oxidation was greater than that of individual compounds. This protective influence of spice principles was also indicated by the relative anodic electrophoretic mobility of oxidized LDL on agarose gel. It has been suggested that dietary curcumin and capsaicin individually are protective to LDL oxidation both in vivo and in vitro, to iron-induced

hepatotoxicity . This beneficial effect was higher when the two compounds were fed in combination.

Kempaiah and Srinivasan, (2004) confirmed that dietary hypolipidemic spices (curcumin, capsaicin and garlic) were effective in reducing the oxidant stress, which was indicated by countering the depleted antioxidant molecules and antioxidant enzymes in erythrocytes and liver, and decreasing the elevated lipid peroxide content.

Cholesterol lowering effect of clove and cardamom was reported by EL-Segay et al ., (2007) who showed that ethanol feeding caused elevation of serum liver enzymes and serum total lipid, total cholesterol and triglyceride levels. Also, there was significant increase in lipid peroxidation product, malonaldehyde (MDA), and decrease in antioxidant enzyme, trace element levels in liver homogenate. On the other hand, the hepatoprotective effect of cardamom and clove was reflected by the significantly lower level of liver enzymes and serum lipid profile in rats pretreated with their extract before ethanol. On the other hand, MDA level was significantly reduced as compared to ethanol fed group, whereas, levels of SOD, and GSH-Rd activity and trace element level were significantly increased by clove and cardamom pretreatment. El-Segaey et al., (2007) reported decreased serum triglyceride and cholesterol levels in cardamom and clove pretreated rats reflecting their protective hepatocellular effects. Furthermore, it has been reported that spices may inhibit hepatic -HMG-CoA reductase activity , resulting in lowering hepatic and serum cholesterol levels. Shobana and Naidu. (2001). Clove was classified by Sharma et al., 2001 as a source for powerful antioxidant activity, whereas, cardamom had a medium level antioxidant power, in addition to its effectiveness in reducing LDL susceptibility to oxidation (Nair et al., 1998). Shamee et al ., (2007) found that Aqueous suspensions of cinnamon and cardamom enhance the level of detoxifying enzyme (GST activity) with simultaneous decrease in lipid peroxidation levels in the treatment groups when compared to that of the carcinogen control group.

There is a significant increase ($p < 0.05$) of serum MDA and liver iron deposition in group 2 and the significant decrease ($p < 0.05$) in serum catalase are in agreement with similar finding by Whittaker and Chanderbhan ., (2001) who reported that feeding rats diets supplemented with carbonyl Fe resulted in dose-related increases in liver nonhaem Fe and lipid peroxidation. EL-Maraghy et al. (2009) demonstrated that administration of FeNTA induced a significant deposition of iron in rat liver associated with exacerbated oxidative stress status and a remarkable increase in hepatic nitric oxide level.

A possible mechanism for tissue damage from chronic Fe overload may be the effect of free radical formation and lipid peroxidation (Whittaker and Chanderbhan, 2001). Whittaker et al. (1996b) previously reported that liver lipid peroxides were significantly increased in Fe-overloaded rats.

Supplementation with either 2% turmeric , clove, chili, or cardamom significantly decreased ($p < 0.05$) serum MDA and liver iron deposition compared to iron overloaded group with the lowest value in group 4 fed 2% clove . On the other hand, a significant increase ($p < 0.05$) in serum catalase in groups supplemented with different spices was reported with the highest value in group 4 fed 2% clove .

Several studies have demonstrated that curcumin can bind iron and it has properties of an iron chelator (Jiao et al., 2006; Messner et al., 2009).

These results are in line with those of Manjunatha and Srinivasan, (2006) who reported that dietary curcumin, capsaicin and their combination reduced the activities of elevated serum enzymes, ALT, AST and LDH which were elevated by iron injection, and lowered the liver lipid peroxide, indicating amelioration of the severity of iron-induced hepatotoxicity. Oboh et al., (2007) concluded that hot peppers prevent Fe^{2+} -induced lipid peroxidation.

Pulla Reddy and Lokesh , (1996) reported that oral administration of spice principles, curcumin from turmeric (30 mg/kg body weight) or eugenol from cloves (100 mg/kg body weight), for 10 days lowered the liver and serum lipid peroxide levels. It has been shown that curcumin reduced iron-dependent oxidative stress and iron toxicity in T51B cells without blocking iron uptake (Messner et al ., 2009) Thephinlap et al ., (2009) found that , Curcuminoids are effective in chelation of plasma NTBI in iron-loaded thalassemic mice. Consequently, it can alleviate iron toxicity and harmfulness of free radicals.

Our results agreed with Srichairatanakool et al ., (2007) who found that curcumin acts as an iron chelator, mice that were fed diets supplemented with curcumin exhibited a decline in levels of ferritin protein in the liver. These results suggest that iron chelation may be an additional mode of action of curcumin.

Lipid peroxidation catalyzed by soybean lipoxygenase was inhibited by eugenol in a concentration-dependent manner. The inhibitory mechanism implies that eugenol does not inactivate the enzyme directly but may interfere with fatty acid radical intermediates due to its hydroxy radical scavenging ability and thus play a role in inhibiting the propagation of lipid peroxidation (Naidu , 1995).

Curcumin and eugenol also prevented the oxidation of Fe²⁺ in Fenton's reaction which generates .OH radicals. (Reddy and Lokesh ., 1994).

Fu, et al., 2008 demonstrated that, curcumin attenuates oxidative stress by increasing the content of hepatic glutathione, leading to the reduction in the level of lipid hydroperoxide.

It has been reported that In in vitro experiments curcumin inhibited iron catalyzed lipid peroxidation in liver homogenates, scavenged nitric oxide spontaneously generated from nitroprusside and inhibited heat induced hemolysis of rat erythrocytes. (Naik, et al., 2010).

Dairam et al. (2008) investigated the antioxidant and metal-binding properties of curcumin, capsaicin, and S-allylcysteine, which are major components found in commonly used dietary spice ingredients turmeric, chili, and garlic, respectively. They demonstrated that these compounds readily scavenge free radicals. These compounds significantly curtail iron- (Fe²⁺) and quinolinic acid (QA)-induced lipid peroxidation. The ferrozine assay demonstrated that these compounds bind Fe²⁺ and Fe³⁺ and prevent the redox cycling of iron, suggesting that this may be an additional method through which these agents reduce Fe²⁺-induced lipid peroxidation.

Nagababu et al. (2010) reported that, Eugenol (4-allyl-2 methoxyphenol) is one such naturally occurring phenolic compound. The antioxidant activity of eugenol was evaluated by the extent of protection offered against free radical-mediated lipid peroxidation using both in vitro and in vivo studies. Eugenol completely inhibited both iron and Fenton reagent-mediated lipid peroxidation. The inhibitory activity of eugenol was about five fold higher than that observed for α -tocopherol.

It has been reported that the antioxidant activity of clove and cardamom could be attributed to its phytochemical contents which increases the amount or increase the activity of antioxidant enzymes (Rock et al ., 1996), or due to their trace element contents which are required for the antioxidant enzyme activity (Lamp (1999; Sharma et al ., 2001).

As previously reported by Khan ., 2003 analysis of clove and cardamom extracts demonstrated that they contain copper and manganese while selenium and zinc are absent. Copper and manganese are required for superoxide dismutase enzyme. Their presence in clove and cardamom extracts stimulated superoxide dismutase which is an antioxidant enzyme leading to decreased lipid peroxidation in rats pre-fed with them.

Acharya et al., (2010) reported that, phytochemicals in cardamom have not been explored

in great details but limonene and cineole demonstrate promising effects against carcinogenesis.

5. Conclusion:

The observed improvement in the liver functions, lipid profile and antioxidant stress in iron-overloaded rats suggests that the chemo-protective effect of the turmeric, clove, chili and cardamom is attributed to chelation with iron followed by excretion of the complex. This result may find application among populations at risk of iron overload, either acquired or inherited.

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