Journal of Medicinal Plants Research Vol. 5(3), pp. 456-461, 4 February, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Compensatory effects of curcumin on cisplatin-induced toxicity in rabbit testis

F. M. Kandemir[1]*, F. Benzer[2], N. C. Yildirim[3] and N. Ozdemir[1]

[1]Department of Biochemistry, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey.
[2]Veterinary Control and Research Institute, Elazig, Turkey.
[3]Department of Environmental Engineering, Faculty of Engineering, Tunceli University, Tunceli, Turkey.

Accepted 20 December, 2010

Curcumin, a widely used spice and colouring agent in food, has been shown to possess potent antioxidant, antitumor promoting and anti-inflammatory properties *in vitro* and *in vivo*. The present study was designed to investigate the protective effects of curcumin on changes in the levels of lipid peroxidation and endogenous antioxidants induced by cisplatin (cis-diamminedichloroplatinum II, CDDP) in the testiclular tissue of rabbits. 18 healthy male New Zealand white rabbits were equally divided into three groups of six rabbits each, control, cisplatin, and cisplatin+curcumin. The degree of protection produced by cisplatin was evaluated by determining the level of malondialdehyde (MDA) and glutathione (GSH), the activity of catalase (CAT), glutathione peroxidase (GSH-Px), were estimated from testes homogenates. MDA levels were increased with cisplatin compared to control but in cisplatin+curcumin group, MDA levels were found to be lower than cisplatin group ($p<0.05$). The activity of CAT and GSH-P_X was decreased in cisplatin and cisplatin+curcumin groups compared to control ($p<0.05$). In the case of cisplatin+curcumin CAT and GSH-Px activity were increased compared to cisplatin group ($p<0.05$). GSH levels were decreased with cisplatin but administration cisplatin+curcumin increased the levels of GSH comared to cisplatin group ($p<0.05$). In the present study, co-administration of curcumin with cisplatin prevented the damage to testes induced by this drug and may be considered as a potentially useful candidate in the combination chemotherapy with cisplatin.

Key words: Rabbit, cisplatin, curcumin, testes, oxidative stress.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum- II) is a widely prescribed anticancer drug. Activity has been demonstrated against a variety of neoplasm's, particularly in the head and neck, testis and ovary, bladder and small-cell lung cancers. High doses of cisplatin can damage different tissues such as kidney, liver and testes (Atessahin, et al., 2006).

Oxidative stress is generally considered as an imbalance between prooxidant/antioxidant (Lieber, 1997). When antioxidant defences are impaired or overcome, oxidative stress may produce DNA damage, enzymatic inactivation and peroxidation of cell constituents, especially lipid peroxidation (Halliwell and Gutteridge, 1989). MDA is used as marker of oxidation of membrane phospholipids through lipid peroxidation (Charissou et al., 2004). Intake of cisplatin results in excessive generation of free radicals, which alter the bio membranes and cause severe damage. Endogenous protection against oxidative stress is achieved by enzymes that catalytically remove free radicals and other reactive species. These includes: superoxide dismutase, catalase and glutathione peroxidase (Faria et al., 2007). It has not commonly been

*Corresponding author. E-mail: fmk_03@mynet.com. Tel: +90 4242370000. Fax: +90 4242338720.

Abbreviations: CDDP, cis-diamminedichloroplatinum II; **MDA,** malondialdehyde; **GSH,** glutathione; **CAT,** activity of catalase; **GSH-Px,** glutathione peroxidases; **ROS,** reactive oxygen species.

used as a therapeutic agent because of its nephrotoxicity risk. The underlying mechanism in nephrotoxicity has been attributed to reactive oxygen species (ROS) (Gulec et al., 2004). ROS is a recently recognized mechanism in the pathogenesis of the CIS-induced testicular toxicity in experimental studies (Atessahin et al., 2006; Turk et al., 2008).

Cisplatin causes lipid peroxidation (LPO) and decreases the activity of enzymes that protect against oxidative damage in testicular tissue from cisplatin-treated rats (Atunes et al., 2001; Silva et al., 2001).

Curcumin is an active constituent of *Curcuma longa* L., *Curcuma aromatica* SALISB. and *Curcuma zedoaria* (BERG.) ROSC, which were used in clinical Chinese medicine as aromatic stomachic, choleretic and for the treatment of menstruation irregularity. In recent decades, pharmacological studies reported that curcumin possessed various promising biological activities: hypocholesteremic (Patil and Srinivasan, 1971), anti-inflammatory (Rao et al., 1982; Satoskar et al., 1986) anti-platelet (Srivastava et al., 1986), antioxidant (Masuda et al., 1999), cancer chemopreventive (Mariadason et al., 2000), anticancer (Chan, 1995; Singh and Aggarwal, 1995), antimutagenic (Nagabhushan et al., 1987), and anti-HIV (Jordan and Drew, 1996), etc.

Sreejayan and Rao (1994) claimed that the presence of phenolic groups in the structure of curcumin is fundamental in explaining its ability to eliminate oxygen-derived free radicals from the medium largely responsible for the peroxidation of cell lipids. They are able mainly to eliminate the hydroxyl radical (Reddy and Lokesh, 1994), superoxide radical (Sreejayan and Rao, 1996), singlet oxygen (Rao et al., 1995), nitrogen dioxide (Unnikrishnan and Rao, 1995), and nitric oxide (Rao, et al., 1997). It has also been demonstrated that curcumin inhibits the generation of the superoxide radical (Ruby et al., 1995).

The present work aimed to evaluate the protective effect of curcumin against cisplatin-induced testicular damage and oxidative stress in rabbit.

MATERIALS AND METHODS

Animals

18 healthy male New Zealand white rabbits, weighing 2.5 to 3 kg, were used in this study. The animals were obtained from the Veterinary Control and Research Institute, Elazig, Turkey. The animals were kept under standard laboratory conditions (12- h light:12- h dark and 24±3°C). The rabbits were fed with standard commercial rabbit chow (pellet form, in the sack, Elazig Food Company). Feed and water were provided ad libitum. The protocol of this study was approved by the Veterinary Control and Research Institute Ethics Committee.

Study design and treatment

Cisplatin (50 mg/100 ml, Code 1876A) was purchased from faulding pharmaceuticals Pic (Warwickshire, UK). Curcumin was kindly provided by Merck (Catalog number 820354).

The rabbits were randomly divided into three groups; each group containing six rabbits. The first group of rabbits served as control and was administered a single intraperitoneal dose of 0.9% saline. The second group of rabbits was treated with cisplatin. Cisplatin was intraperitoneally injected to animals at a single dose of 5 mg/kg body weight. The third group of rabbits was treated with curcumin animals by gavage in corn oil at the dose of 100 mg/kg body weight) for 6 consecutive days before and 6 consecutive days after a single intraperitoneal dose of 5 mg/kg body weight cisplatin injection.

Lipid peroxidation level

At the end of the experiment, the rabbits were decapitated under slight ether anaesthesia. Testicular tissue was removed and homogenized in a glass-glass homogenizer with a buffer containing 1.5% potassium chloride to obtain 1:10 (w/v) whole homogenate. MDA, which formed as a final product of the peroxidation of lipids, served as an index of the intensity of oxidative stress. MDA, referred to as thiobarbituric acid reactive substance, was measured with thiobarbituric acid at 532 nm in a spectrophotometer, as described previously (Placer et al., 1966). The MDA level was expressed as nmol/g wet tissue.

Glutathione peroxidase activity

GSH-Px (EC 1.11.1.9) activity was determined using cumene hydroperoxide and reduced GSH as co-substrates and the loss of GSH following enzymatic reaction was measured spectrophotometrically with Ellman's reagent at 37°C and 412 nm according to Lawrence and Burk (1976).

Catalase activity

The testicular tissue catalase activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, according to the method of Aebi (1983) and was expressed as k/g protein, where *k* is the first-order rate constant.

GSH level

Reduced GSH was estimated by the method of Sedlak and Lindsay (Sedlak and Lindsay, 1968), where the colour developed was read at 412 nm. Protein concentrations in all samples were measured using the method of Lowry et al. (1951). Results were reported as nmol/g protein.

Statistical analysis

All values are presented as mean ± S.E.M. All groups were compared by one-way analyses of variance (ANOVA) and post hoc multiple comparisons were done with Duncan test in SPSS/PC software program (version 12.0; SPSS Inc., Chicago, IL, USA) to determine the differences in all parameters.

RESULTS AND DISCUSSION

The testes MDA levels were significantly increased in cisplatin treated group when compared to control ($p<0.05$). In cisplatin+curcumin group, MDA levels were

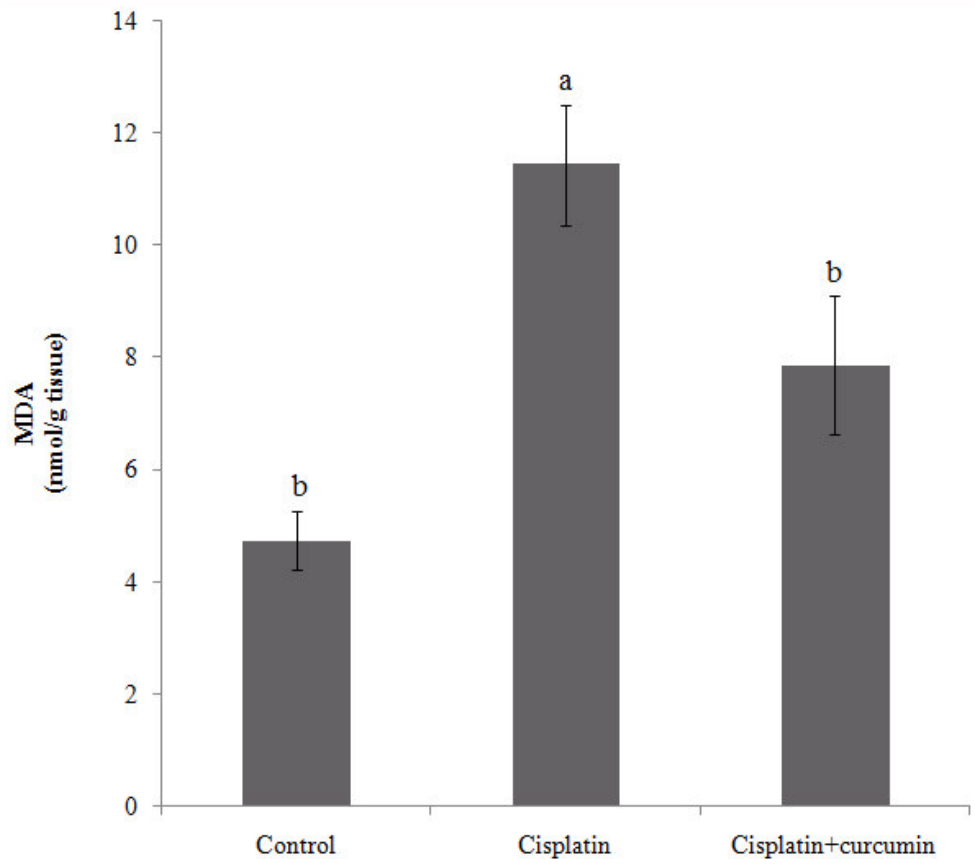

Figure 1. Effects of curcumin on MDA levels under cisplatin-induced oxidative stress conditions in rabbits, different letters on top of bars indicate statistically importance according to Duncan's Multiple Range test ($p<0.05$).

increased when compared to control but this increase was not significant statically ($p>0.05$). Administration cisplatin and curcumin decreased the MDA levels of testes when compared to cisplatin group ($p<0.05$) (Figure 1). CAT and GSH-P_X activity were decreased depending on cisplatin and cisplatin+curcumin administration compared to control ($p<0.05$). In cisplatin+curcumin group CAT ($p>0.05$) and GSH-P_X ($p<0.05$) activity were increased when compared to cisplatin group (Figures 2 and 3).

In cisplatin group, GSH levels were decreased but in cisplatin+curcumin group were increased compared to control ($p<0.05$). GSH levels were increased with administration of cisplatin+curcumin compared to cisplatin group ($p<0.05$) (Figure 4).

Curcumin, a widely used spice and colouring agent in food, has been shown to possess potent antioxidant, antitumor promoting and anti-inflammatory properties *in vitro* and *in vivo* (Motterlini et al., 2000). Many studies have shown the capacity of curcumin to prevent lipid peroxidation, a key process in the onset and progression of many diseases (Venkatesan, 2000).

In recent years, several studies highlighted the ability of curcumin to promote a variety of pharmacological and biological activities (Ammon and Wahl, 1991). For instance, by virtue of its flavonoid chemical structure, this yellow pigment appears to possess antioxidant and free radical-scavenging characteristics. Curcumin, in fact, neutralizes active oxygen species including superoxide, hydroxyl radical and nitric oxide (Kunchandy and Rao, 1990). In renal epithelial cells, curcumin has been reported to inhibit lipid peroxidation resulting in protection against the cytotoxic action of hydrogen peroxide (Cohly et al., 1998). The antioxidant mechanism of curcumin may include one or more of the following interactions. Scavenging or neutralizing of free radicals, interacting with oxidative cascade and preventing its outcome, oxygen quenching and making it less available for oxidative reaction, inhibition of oxidative enzymes like cytochrome P450 and chelating and disarming oxidative properties of metal ions such as iron (Rukkumani et al., 2004).

Motterlini et al. (2000) indicate that curcumin is a potent inducer of HO^{-1} in vascular endothelial cells and that increased heme oxygenase activity is an important component in curcumin-mediated cytoprotection against

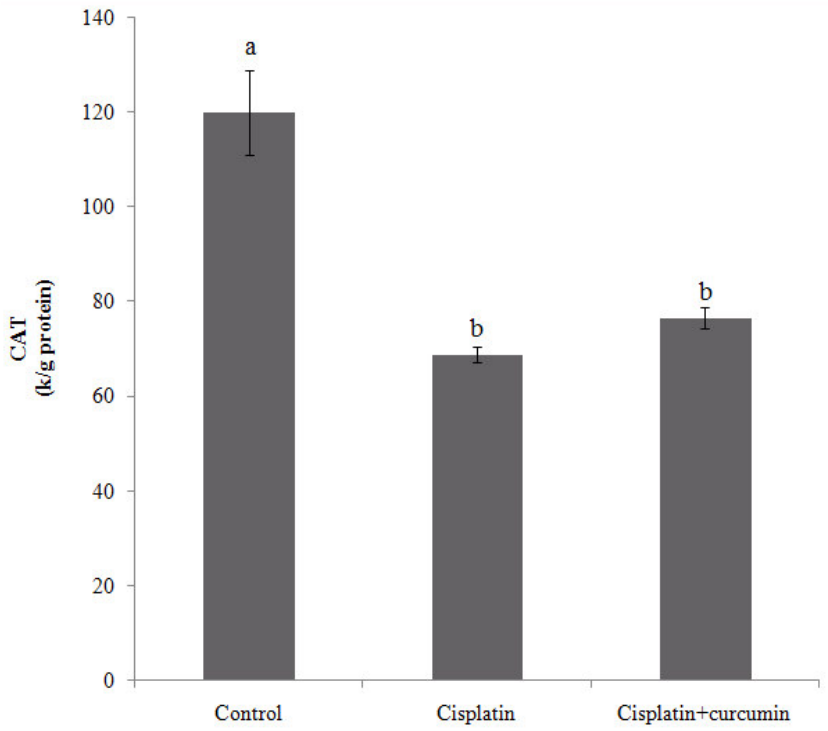

Figure 2. Effects of curcumin on CAT activity under cisplatin-induced oxidative stress conditions in rabbits, different letters on top of bars indicate statistically importance according to Duncan's Multiple Range test ($p<0.05$).

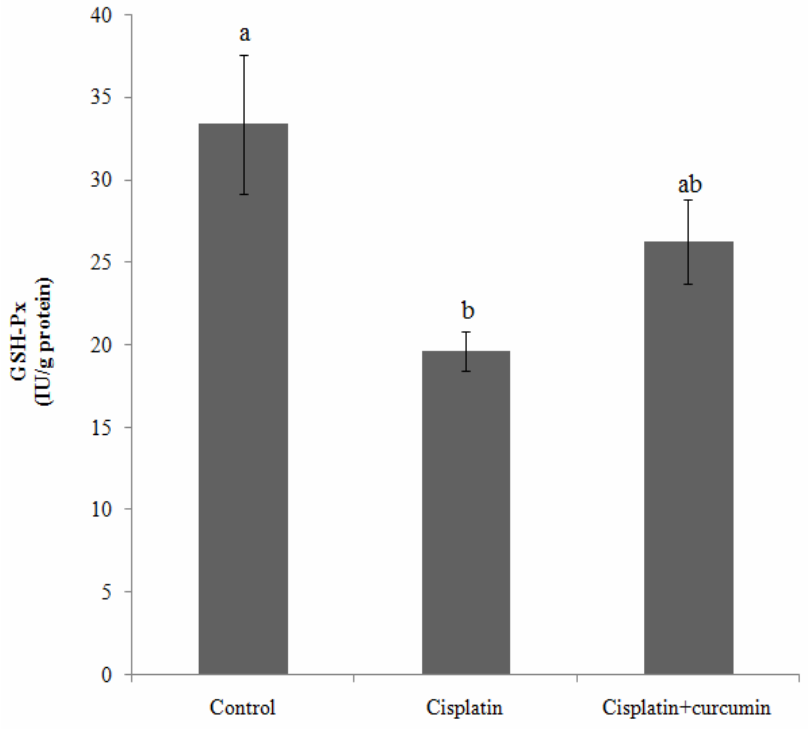

Figure 3. Effects of curcumin on GSH-Px activity under cisplatin-induced oxidative stress conditions in rabbits, different letters on top of bars indicate statistically importance according to Duncan's Multiple Range test ($p<0.05$).

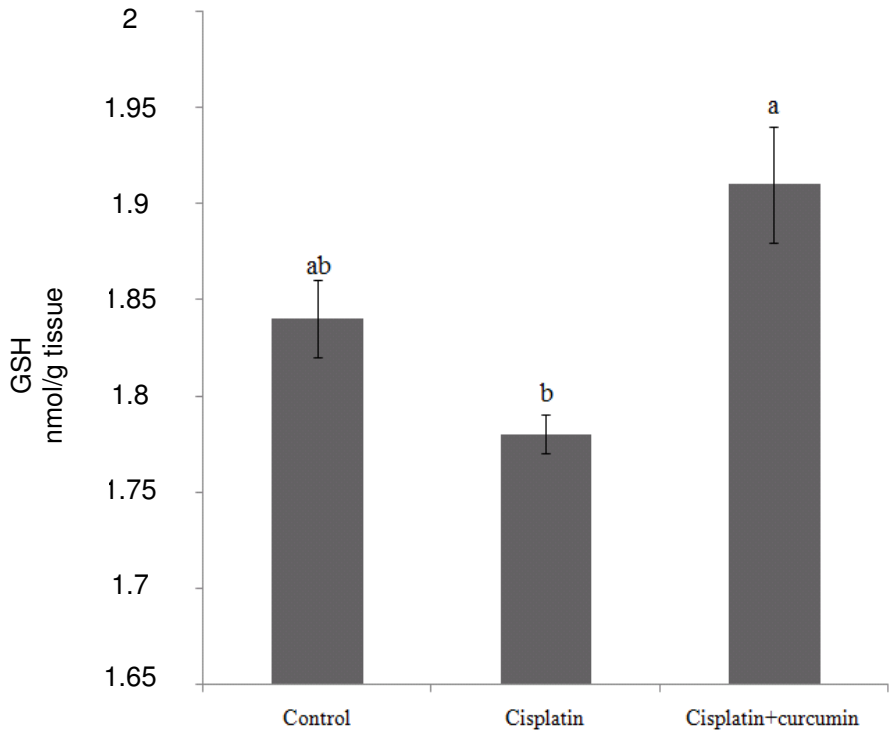

Figure 4. Effects of curcumin on GSH levels under cisplatin-induced oxidative stress conditions in rabbits, different letters on top of bars indicate statistically importance according to Duncan's Multiple Range test ($p<0.05$).

oxidative stress. Agarwal et al. (2010) suggest that curcumin pretreatment has a protective effect and that curcumin can be used as a therapeutic agent in mercury intoxication. The study indicates that curcumin, an effective antioxidant, may have a protective effect through its routine dietary intake against mercury exposure. Okada et al. (2001) demonstrated that curcumin and especially tetrahydrocurcumin ameliorate oxidative stress-induced renal injury in mice.

Kawluru et al. (2007) examined the effect of curcumin, a polyphenol with antioxidant and anti-inflammatory properties, on diabetes-induced oxidative stress and inflammation in the retina of rats. They suggested that curcumin could have potential benefits in inhibiting the development of retinopathy in diabetic patients. Tirkey et al. (2005) suggested that curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. Oxidative damage caused by ROS has been implicated in the pathogenesis of cisplatin-induced testicular injury (Ilbey et al., 2009).

In consistent with these reports, our results showed that the administration of cisplatin resulted in a significant reduction in testis GSH and GSH-Px, CAT activity and elevated MDA compared with the untreated control animals. It was observed that cisplatin induced negative effects in antioxidant enzymes including GSH peroxidase, catalase activities and GSH, MDA levels were prevented by curcumin compared to the cisplatin alone group. This is the first report showing that curcumin, a polyphenol, has beneficial effects on cisplatin-induced oxidative stress in testes tissue of rabbits. This protective effect of curcumin seems to be closely involved with the suppression of oxidative stress.

Curcumin has been considered to be mediated via its beneficial effects on the antioxidant defense system, the scavenging of free radicals and/or via preventing lipid peroxidation. Results from this study indicate that the novel natural antioxidant curcumin might have protective effect against cisplatin-induced testicular damage and oxidative stress in rabbit.

REFERENCES

Aebi H (1983). Catalase. In: HU Bergmeyer (ed.). Methods in Enzymatic Analysis. Academic Press, New York, pp. 276–286.

Agarwal R, Goel SK, Behari JR (2010). Detoxification and antioxidant effects of curcumin in rats experimentally exposed to mercury. J. Appl. Toxicol., 30(5): 457-468.

Ammon HPT, Wahl MA (1991). Pharmacology of *Curcuma longa.* Planta. Med., 57: 1–7.

Antunes LM, Darin JD, Bianchi NDE L (2001). Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res., 43: 145–150.

Atessahin A, Karahan I, Turk G, Gür S, Yilmaz S, Ceribasi AO (2006). Protective role of lycopene on cisplatin-induced changes in sperm characteristics, testicular damage and oxidative stress in rats.

Reprod. Toxicol., 21: 42–47.
Chan MM (1995). Inhibition of tumor necrosis factor by curcumin, a phytochemical. Biochemical. Pharmacol., 49: 1551-1556.
Charissou AM, Cossu-leguille C, Vasseur P (2004). Relationship between two oxidative stress biomarkers, malondialdehyde and 8-oxo-7,8-dihydro-2'-deoxyguanosine, in the freshwater bivalve Unio tumidus. Sci. Total Environ., 322: 109–122.
Cohly HHP, Taylor A, Angel MF, Salahudeen AK (1998). Effect of turmeric, turmerin and curcumin on H2O2-induced renal epithelial (LLC-PK1) cell injury. Free Radic. Biol. Med., 24: 49–54.
Faria A, Monteiro R, Mateus N, Azevedo I, Calhau C (2007). Effect of pomegranate (*Punica granatum*) juice intake on hepatic oxidative stress. Eur. J. Nutr., 46: 271–278.
Gulec M, Yilmaz HR, Iraz M, Aglamis S, Sogut S (2004). The effects of *Ginkgo biloba* extract on plasma glutathıon peroxıdase, superoxıde dismutase, adenosine deaminase and nitric oxide levels in cisplatin-induced nephrotoxicity, J. Med. Sci., 24: 585-591.
Halliwell B, Gutteridge JMC (1989). Free radicals in biology and medicine, 543 pages, 2nd ed. Clarendon Press, Oxford.
Ilbey YO, Ozbek E, Cekmen M, Simsek A, Otunctemur A, Somay A (2009). Protective effect of curcumin in cisplatin-induced oxidative injury in rat testis: mitogen-activated protein kinase and nuclear factor-kappa B signaling pathways. Hum. Reprod., 24(7): 1717–1725.
Jordan WC, Drew CR (1996). Curcumin - a natural herb with anti- HIV activity. Natl. Med. Assoc., 88: 333.
Kowluru RA, Kanwar M (2007). Effects of curcumin on retinal oxidative stress and inflammation in diabetes. Nutr. Metabol., 4: 8.
Kunchandy E, Rao MNA (1990). Oxygen radical scavenging activity of curcumin. Int. J. Pharm., 58: 237–240.
Lawrence RA, Burk RF (1976). Glutathione peroxidase activity in selenium-deficient rat liver. Bioch. Biophys. Res Commun., 71: 952-958.
Lieber CS (1997). Ethanol metabolism, cirrhosis and alcoholism. Clin. Chim. Acta, 257: 59-84.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
Mariadason JM, Corner GA, Augenlicht LH (2000). Genetic reprogramming in pathways of colonic cell maturationinduced by short chain fatty acids: comparison with trichostatin a, sulindac, and curcumin and implications forchemoprevention of colon cancer. Cancer. Res., 60: 4561-4572.
Masuda T, Hidaka K, Shinohara A, Maekawa T, Takeda Y, Yamaguchi HJ (1999). Chemical studies on antioxidant mechanism of curcuminoid: analysis of radical reaction products from curcumin. Agric. Food Chem., 47: 71-77.
Motterlini R, Foresti R, Bassi R, Gren CJ (2000). An antioxidant and anti-inflammatory agent induces heme oxygenase-1 and protects endothelial cells against oxidative stress. Free Radic. Biol. Med., 28: 1303–1312.
Nagabhushan M, Amonkar AJ, Bhide SV (1987). *In vitro* antimutagenicity of curcumin against environmental mutagens. Food Chem. Toxicol., 25: 545-547.
Okada K, Wangpoengtrakul C, Tanak T, Toyokuni S, Uchida K, Osawa T(2001). Curcumin and especially tetrahydrocurcumin ameliorate oxidative stress-induced renal injury in mice. J. Nutr., 131: 2090-2095.
Patil TN, Srinivasan M (1971). Hypocholesteremic effect of curcumin in induced hypercholesteremic rats. Indian. J. Exp. Biol., 9: 167-169.
Placer ZA, Cushmann LL, Johnson BC (1966). Estimation of products of lipid peroxidation (as malondialdehyde) in biochemical systems. Anal. Biochem., 16: 359-364.
Rao CV, Rivenson A, Simi B, Reddy BS (1995). Chemoprevention of colon carcinogenesis by dietary curcumin, a naturally occurring plant phenolic compound. Cancer. Res., 55: 259–266.
Rao TS, Basu N, Siddigui HH (1982). Anti-inflammatory activity of curcumin analogues. Indian J. Med. Res., 75: 574-578.
Reddy AC, Lokesh BR (1994). Studies on the inhibitory effects of curcumin and eugenol on the formation of reactive oxygen species and the oxidation of ferrous iron. Mol. Cell. Biochem., 137: 1–8.
Ruby AJ, Kuttan G, Babu KD, Rajasekharan KN, Kuttan R (1995). Anti-tumor and antioxidant activity of natural curcuminoids. Cancer Lett., 94: 79–83.
Rukkumani R, Aruna K, Suresh VP, Rajasekaran KN, Menon VP (2004). Comparative effects of curcumin and an analog of curcumin on alcohol and pufa induced oxidative stress. J. Pharm. Pharmaceut. Sci., 7: 274-283.
Satoskar RR, Shah SJ, Shenoy SG (1986). Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with postoperative inflammation. Int. J. Clin. Pharmcol. Ther. Toxicol., 24: 651-654.
Sedlak J, Lindsay RHC (1968). Estimation of total protein bound and nonprotein sulfhydryl groups in tissue with Ellmann's reagent. Anal. Biochem., 25: 192-205.
Silva CR, Greggi Antunes LM, Bianchi ML (2001). Antioxidant action of bixin against cisplatin-induced chromosome aberrations and lipid peroxidation in rats. Pharmacol. Res., 43: 561–566.
Singh S, Aggarwal BB (1995). Activation of transcription factor NF-kappa B is suppressed by curcumin (diferuloylmethane). *J. Biol. Chem.,* 270: 24995-25000.
Sreejayan N, Rao MNA (1994). Curcuminoids as potent inhibitors of lipid peroxidation. J. Pharm. Pharmacol., 46: 1013–1016.
Sreejayan N, Rao MNA (1996). Free radical scavenging activity of curcuminoids. Arzneimittelforschung., 46: 169–171.
Srivastava R, Puri V, Srimal RC, Dhawan BN (1986). Effect of curcumin on platelet aggregation and vascular prostacyclin synthesis. Arzneim.-Forsch./Drug Res., 36: 715-717.
Tirkey N, Kaur G, Vij G, Chopra K (2005). Curcumin, a diferuloylmethane, attenuates cyclosporine-induced renal dysfunction and oxidative stress in rat kidneys. BMC Pharmacol., 5: 15.
Turk G, Atessahin A, Sonmez M, Ceribas AO, Yuce A (2008). Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil. Steril., 89: 1474–1481.
Unnikrishnan MK, Rao MNA (1995). Curcumin inhibits nitrogen dioxide induced oxidation of hemoglobin. Mol. Cell. Biochem., 146: 35–37.
Venkatesan N (2000). Pulmonary protective effects of curcumin against paraquat toxicity. Life Sci., 66: 21–28.