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RESEARCH ARTICLE

DEFENSIVE ROLE OF INDIAN SPICE CURCUMIN AGAINST NICOTINE INDUCED DAMAGE ON LUNG TISSUE IN MALE ALBINO RAT

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ABSTRACT

Curcumin is a yellow colouring ingredient of the spice turmeric obtained from the rhizome of Curcuma longa Linn (Zingiberaceae). It has a wide spectrum of biological and pharmaceutical activities such as anti-inflammatory, anti-fungal, antiviral, anti-mutagenic, and anti-carcinogenic and anti-oxidant activities. Nicotine is the principal alkaloid contained in tobacco,nicotine metabolism has been shown to occur to a small extent in extrahepatic organs such as lung, kidney and brain. Pathogen free, Wistar strain male albino rats were used in the present study, Age matched rats were be divided into 4 groups of six in each group and treated as follows: Group I. Normal Control (NC) (Control rats received 0.9% saline). Group II. Nicotine treated (Nt) (at a dose of 0.6 mg/ kg body weight by subcutaneous injection for a period of 2 months). Group III. Rats will receive the nicotine with a dose of 0.6 mg/kg body weight by subcutaneous injection and Curcumin extract 50mg/kg body weight (after the standardization) via orogastric tube for a period of 2 months. The animals were sacrificed after 24 hours after the last treatment by cervical dislocation and isolated the lung tissue washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -800 C for biochemical analysis and enzymatic assays. In the present study the Malondialdehydehyde (MDA), were significantly increased in nicotine treated rats in the lung tissue and reduced was observed in the combination treatment (Nt+Cur), Whereas Glutathione s-transferase, Xanthion oxidase (XOD), Cytochrome-C- oxidase and Glucose-6-phosphate dehydrogenase were considerably decreased in nicotine treated rats meanwhile In all the experimental animals upregulated of enzymes was observed in the lung tissue (combination treatment of Nt+Cur). The present study has been undertaken to evaluate the antioxidant property of Curcumin on lung tissue, Keeping in view of the relative importance of Curcumin on nicotine induced oxidative stress in male albino rats.

INTRODUCTION

The spice turmeric that obtained from Curcuma longa Linn (Zingiberaceae). It possesses anti-inflammatory, immunomodulatory, and antiatherogenic activities and is a potent inhibitor of various reactive oxygen-generating enzymes. Curcumin is a potent scavenger of reactive oxygen species including superoxide anion radicals and hydroxyl radicals (Chiagoziem *et al*., 2014). Curcumin is a β-diketone compound which contains two ferulic acid molecules linked via a methylene bridge at the carbon atoms of the carbonyl groups (Sharma OP, 1979) Various curcumin related phenols (Curcuminoids) have also been found in edible plants, especially Zingiberaceae plants. Extracts of rhizomes of turmeric have been widely used in Indian medicine and they are considered to be efficacious in the treatment of liver disorders and certain pyrogenic infections (Nadakarni AK 1954).

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Curcumin exhibits anti inflammatory (Joe B, Vijaykumar M, Lokesh B.2004). It has also been reported to inhibit lipid peroxidation (Borra *et al*., 2013). Curcumin administration attenuated oxidative stress in rats. It also prevented free radical formation-induced many disorders and protected against many induced toxicity (Messarah *et al*., 2013). Scientists have been focusing on chemopreventive approach to ameliorate the effect of nicotine toxicity using natural compounds, particularly polyphenols, many of which are gifted with excellent chemopreventive properties, that has been reported to possess anti-inflammatory and antioxidant properties (Suzuki *et al.,* 2009). Turmeric (Curcumalanga), one of the oldest plants, belongs to the family of Zingiberaceae family which has long been used in traditional medicine for blood purification, digestion, arthritis treatment, liver protection and as an anti-inflammatory agent. (Agarwal *et al.,* 2004). Nicotine is a highly toxic organic compound containing nitrogen and alkaloid which is mostly found in tobacco (Jana *et al*., 2010). Nicotine can easily pass through the cell membrane and react to tubulin protein present in the cytoplasm of multiplying cells and cause cell division disorder (Gorrod, 1993). Previous

studies have indicated that nicotine can damage sperm membrane and DNA and induce apoptosis in interstitial cells in testis. Nicotine also induces oxidative stress both in *vivo* and in *vitro* that causes a peroxidant/antioxidant imbalance in blood cells, blood plasma and tissues (Suleyman *et al*., 2002). Nicotine is metabolized primarily in the liver. In animals, nicotine metabolism has been shown to occur to a small extent in extrahepatic organs such as lung, kidney and brain (Gorrod and Jenner, 1975; Williams *et al*., 1990a; Vahakangas and Pelkonen, 1993; Jacob *et al*., 1997). Smoking a cigarette delivers nicotine rapidly to the pulmonary venous circulation, from which it moves quickly to the left ventricle of the heart and to the systemic arterial circulation and to the brain. Although the delivery of nicotine to the brain is rapid, there is nevertheless significant pulmonary uptake and some delayed release of nicotine as evidenced by pulmonary positron emission tomography data and the slow decrease in the arterial concentrations of nicotine between puffs (Rose *et al*., 1999a). Nicotine is the primary psychoactive substance that reinforces smoking behaviour (Henningfield and Goldberg, 1983). Nicotine from inhaled tobacco smoke is rapidly absorbed in the lungs and enters arterial circulation where it is then distributed to various body tissues, distributing in liver, spleen, lungs and brain, with a low affinity for adipose tissue (Hukkanen *et al.,* 2005). Nicotine also demonstrates a preferential partitioning (4 times) in brain over plasma (Ghosheh *et al.,* 2001).

Uptake of nicotine is rapid such there is a 6-10 fold arteriovenous difference in nicotine levels; such rapid delivery to the brain 10-19 seconds, (Benowitz, 1996) is associated with the reinforcing effects of nicotine (Samaha *et al*., 2005). The pharmacokinetics of inhaled tobacco smoke resemble that of intravenous nicotine administration in that high peak levels are obtained rapidly (Henningfield and Keenan, 1993), although there is recent evidence that some nicotine may be temporarily sequestered in the lung before being distributed to the rest of the body (Rose *et al.,* 1999a). The elimination half-life of nicotine in plasma is approximately 2 hours in humans and 50 minutes in rats (Hukkanen *et al.,* 2005). Nicotine is known to induced oxidative stress and depletes antioxidant defense mechanisms and produced reduction in glutathione peroxidase in lung, liver and kidney of nicotine-treated animals (Yildiz, 2004; Muthukumaran *et al*., 2008). Nicotine also increases both free fatty acid release from the liver and the hepatic synthesis of very low-density lipoproteins; also maternal nicotine exposure induced oxidative stress and causes histopathological changes in the lung and liver of lactating offspring (El-Sokkary *et al.,* 2007).

Nicotine has been reported to induce oxidative stress both *in vivo* and *in vitro* (Suleyman *et al.,* 2002).The oxidative stress increases in smokers and in patients with Chronic Obstructive Pulmonary Disease (COPD). It is known that smoking and chronic bronchitis are both associated with increased numbers of activated neutrophils and macrophages in the airspaces, which release more $O_2^{\cdot-}$ than those from healthy controls (Ludwig and Hoidal, 1982). A correlation between $O_2^{\cdot-}$ release by peripheral blood neutrophils and bronchial hyper reactivity in patients with COPD exists, suggesting a role for ROS in the pathogenesis of the airway abnormalities in COPD. A major site of free radical attack is in polyunsaturated fatty acids in cell membranes producing lipid peroxidation. The end products of lipid peroxidations such as malondialdehyde, ethane and pentane were significantly increased in smokers (Petruzzelli *et al.,* 1990). Oxidative stress may result in over production of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system (Baynes, 1991). The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides (Strain *et al*., 1991). The present study has been undertaken to evaluate the antioxidant property of Curcumin on lung tissue, Keeping in view of the relative importance of Curcumin and their principal focus of this project is aimed at comparative evolution of antioxidant property of Curcumin on nicotine induced oxidative stress in male albino rats.

MATERIALS AND METHODS

Animals

Pathogen free, wistar strain male albino rats were used in the present study. The usage of animals was approved by the Institutional Animal Ethics Committee. The rats were housed in clean polypropylene cages under hygienic conditions with photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard laboratory chow (Hindustan Lever Ltd, Mumbai) and water *ad libitum*.

Procurement of chemicals

All the chemicals used in the present study were Analytical grade (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA)**,** Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India).

Dosage of nicotine

The dose administration of nicotine was followed as per the protocol given by (Shoaib and Stolerman, 1999; Helen *et al*., 2003) 0.6 mg / kg body weight (0.5ml) was chosen as the dose, for this study.

Selection and mode of nicotine treatment

Nicotine was first distilled from tobacco sap in 1809. Nineteen years later, the main base of tobacco was isolated and separated in pure form from fermented as well as non-fermented tobacco by Posselt and Reimann (Pailer, 1964).They called it nicotine and characterized it as a water-clear liquid, boiling under atmospheric pressure at 246^{o}C, miscible with water, alcohol and ether. Historically nicotine had been recommended for treatment of numerous symptoms.

Physical and chemical properties of nicotine

1) Nicotine Scientific name : *Nicotiana tobacco*
2) Nicotine Family : *Solanaceae*
3) Chemical formula : $C_{10}H_{14}N_2$
4) Molecular Weight : 162.23
5) IUPAC Name : 3-[2-(N-methylpyrrolidinyl)]pyridine
6) Appearance : Oily, colourless hygroscopic liquid,
7) Characteristic odour : Turns brown on exposure to air
8) Boiling point (decomposes) : 246 °C
9) Density : 1.01 g cm-3
10) Solubility in water : miscible

Curcumin dose preparation

Curcumin powder ($C_{21}H_{20}O_6$) was purchased from Merk company(Merk-Germany). The powder was dissolved in ethanol 70% (C_2H_5OH) and diluted by normal saline to prepare at a dose of 50mg/kg/day. Curcumin treatment (50mg/kg) revealed no mortalities in any group of wistar male albino rats tested, the compound also had low ulcerogenic index (Srimal RC and Dhawan BN. (1973).

Treatment Schedule

Age matched rats will be divided into 3 groups of six in each group and treated as follows: i) Normal Control, ii) Nicotine, iii) Nicotine + curcumin extract.

Group I – Normal Control: Control rats receive 0.9% saline. **Group II –** Nicotine: Rats will receive the nicotine with a dose of 0.6 mg/kg body weight by subcutaneous injection for a period of 2 months. Group III – Nicotine + Curcumin Extract. Rats will receive the nicotine with a dose of 0.6 mg/kg body weight by subcutaneous injection and Curcumin extract 50mg/kg body weight (after the standardization) via orogastric tube for a period of 2 months.

The animals will be sacrificed after 24 hrs after the last treatment session by cervical dislocation and the lung tissue will be isolated at -4^0C, washed with ice-cold saline, immediately immersed in liquid nitrogen and stored at -80^0C for biochemical analysis and enzymatic assays. Before assay, the tissues will be thawed, sliced and homogenized under ice-cold conditions. Selected parameters will be estimated by employing standard methods.

Biochemical Investigation

In the present study Malondialdehyde (MDA), Glutathione-S-transferase, Xanthion oxidase (XOD), Cytochrome-C oxidase and Glucose-6-phosphate dehydrogenase were analyzed. Malondialdehyde (MDA) activity was determined according to the method of Ohkawa *et al.,* (1979) followed by Glutathione-S-transferase by Habig, (1974), Xanthion oxidase (XOD) by Srikanthan and Krishnamurthi (1955), Cytochrome-C oxidase by Oda *et al*, (1958) and Glucose-6-phosphate dehydrogenase by the method of Lohr and Waller (1965).

Statistical Analysis

The data obtained will be expressed as Mean values with their SD. Using M.S. Office, Excel Software the data will be analyzed for the significance of the main effects (factors), and treatments along with their interactions. Statistical analysis has been carried out using INSTAT software. The data was analyzed for the significance; the results were presented with the P-values.

RESULTS AND DISCUSSION

Cytochrome Activity

The enzyme Cytochrome-C-oxidase or, is a large transmembrane protein complex found in bacteria and the mitochondrion of eukaryotes. It is the last enzyme in the respiratory electron transport chain of mitochondria (or bacteria) located in the mitochondrial (or bacterial) membrane. It receives an electron from each of four cytochrome-c molecules, and transfers them to one oxygen molecule, converting molecular oxygen to two molecules of water. In the process, it binds four protons from the inner aqueous phase to make water, and in addition translocates four protons across the membrane, helping to establish a transmembrane difference of proton electrochemical potential that the ATP synthase then uses to synthesize ATP.

The complex is a large integral membrane protein composed of several metal prosthetic sites and 14 (Balsa E, *et al*., 2012) protein subunits in mammals. In mammals, eleven subunits are nuclear in origin, and three are synthesized in the mitochondria. The complex contains two hemes, a cytochrome a and cytochrome a_3, and two copper centers, the Cu_A and Cu_B centers. (Tsukihara T *et al*.,1995) In fact, the cytochrome a_3 and Cu_B form a binuclear center that is the site of oxygen reduction. Cytochrome c, which is reduced by the preceding component of the respiratory chain (cytochrome bc1 complex, complex III), docks near the Cu_A binuclear center and passes an electron to it, being oxidized back to cytochrome c containing Fe^{3+}. The reduced Cu_A binuclear center now passes an electron on to cytochrome a, which in turn passes an electron on to the cytochrome a_3-Cu_B binuclear center. The two metal ions in this binuclear center are 4.5 Å apart and coordinate a hydroxide ion in the fully oxidized state.

In the present study the significantly decreased (-46.51%) the activities of Cytochrome were observed in the lung tissue of Nicotine treated (Nt) rats when compared to control rats. In all the experimental animals significantly upregulated was observed (Nt+Cur Extract by (- 15.75%) activity of enzyme was bserved in the lung tissue, (Table.1). A significant decline in the levels of cytochrome was noticed in lung tissue of nicotine treated rat as compared to controls. The curcumin treatment showed significant elevation in the cytochrome activity (Table 1). In the present study administration of nicotine altered the cytochrome activity in rat lung tissue. Similar studies revealed that chronic administration of nicotine altered the cytochrome P450 monooxygenase system in rat brain (Hindupur K *et al*.,1993). The induction by dietary nicotine of a series of cytochrome enzyme activities were significantly increased. (Mark J *et al*.,1993). Curcumin is known as antioxidant and anti-inflammatory properties. It is the free radical scavenger and inhibited lipid peroxidation products (Samuhasaneeto, S., D, 2009). The protective mechanism of curcumin may due to the strong antioxidant property i.e. it helped for the healing of hepatic parenchyma and regeneration of hepatocytes. Significantly induces curcumin-mediated release of cytochrome *c* from the mitochondria into the cytoplasm. This curcumin-induced increase in cytochrome *c* within the cytosol is dose-dependent. (Ju-Hyung *et al*., 2003). Similar studies revealed that the inhibition of cytochrome has been also observed in previous animal and human experiences. Gvozdjáková *et al*. (1994) demonstrated a decrease in cytochrome activity of heart muscle mitochondria from rabbits inhaling cigarette smoke, and noted that this decrease was higher with greater length of smoke exposure. Similarly, Örlander and co-workers (Örlander, J, 1979 and Larsson,L. and Örlander, J. 1984) found a decreased cytochrome activity in skeletal muscle mitochondria of smokers, and suggested that tobacco smoke components, especially CO, could be responsible for this decrease. Actually, this hypothesis has been confirmed in

patients suffering from an acute CO poisoning, who develop a severe and persistent inhibition of cytochrome activity (Miró,O,1998).

Table 1. Changes in Cytochrome-C oxidase activity due to Nicotine treatment (Nt), and interaction of the both Nicotine+Curcumin for a period of 2 months over the control in Lung tissue of male albino rats. Values are expressed as micrograms of cytochrome c per gm/ wet wt of tissue

Name of the tissue	Control	Nicotine (Nt)	Nicotine+Curcumin
Lung tissue	42.33 ±10.96	24.135** ±6.58 (-46.51)	35.66* ±7.83 (-15.75)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.

Malondialdehydehyde (MDA)

Malondialdehyde (MDA) is the organic compound with the formula $CH_2(CHO)_2$. The structure of this species is more complex than this formula suggests. This reactive species occurs naturally and is a marker for oxidative stress. Malondialdehyde mainly exists in the enol form: In organic solvents, the *cis*-isomer is favored, whereas in water the *trans*-isomer predominates. Malondialdehyde is a highly reactive compound that is not typically observed in pure form. In the laboratory it can be generated in situ by hydrolysis of 1,1,3,3-tetramethoxypropane, which is commercially available. (V. Nair *et al*., 2008). It is easily deprotonated to give the sodium salt of the enolate (m.p. 245 °C). Malondialdehyde results from lipid peroxidation of polyunsaturated fatty acids. It is a prominent product in Thromboxane A2synthesis wherein cyclooxygenase 1 or cycloxygenase 2metabolizes arachidonic acid to prostaglandin H2 by platelets and a wide array of other cell types and tissues. This product is further metabolized by Thromboxane synthase to Thromboxane. A2, 12-Hydroxyheptadecatrienoicacid, nd malonyldialdehyde.

Alternatively, it may rearrange non-enzymatically to a mixture of 8-cis and 8-trans isomers of 12-hydroxyeicosaheptaenoic acid plus malonyldialdehyde (see 12-Hydroxyheptadecatrienoic acid). The degree of lipid peroxidation can be estimated by the amount of malondialdehyde in tissues. (Davey MW *et al.,* 2005). In the present study a significantly increased (+17.33%) activities of Malondialdehydehyde (MDA) were observed in the lung tissue of Nicotine treated (Nt) rats when compared to normal rats. In all the experimental animals slightly decreased (Nt+Cur Extract) by (+6.33%) activity of enzyme was observed in the lung tissue, (Table 2). The protective role of curcumin in nicotine -induced toxicity in Wistar rats was examined in the present study. Curcumin administration attenuated oxidative stress in rats, (El-Demerdash *et al*., 2009). It also prevented free radical formation-induced many disorders and protected against manyinduced toxicity (El-Maraghy and El-Sawalhi, 2009 and Messarah *et al*., 2013). Administration of nicotine resulted in a significant increase in serum levels of MDA and NO compared to the normal control group. Supplementation of curcumin with Nicotine significant reduction in serum levels of MDA and NO compared to nicotine only treated group. (Mona G *et al*., 2015). In N-acetylcysteine treatment enhancement ameliorated the levels of MDA in plasma and lung as compared to control group (Gurer H *et al*., 1998). According to Zhang J *et al* (2001) Animals studies have shown significantly higher liver and serum levels of MDA, conjugated dienes, hydroperoxides, and free fatty acids in rats induced by cigarette smoke. In the present study an increase in MDA levels reflected nicotine induced oxidative stress.

Table 2. Changes in Malondialdehyde (MDA) activity due to Nicotine treatment (Nt) , and interaction of the both Nicotine+Curcumin for a period of 2 months over the control in Lung tissue of male albino rats. Values are expressed in μ moles of Malondialdehydehyde / gm Wet wt of tissue

Name of the tissue	Control	Nicotine (Nt)	Nicotine+Curcumin
Lung tissue	93.19 ±9.41	109.34** ±9.05 (+17.33)	99.09* ±9.33 (+6.33)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.

Also nicotine caused greater damage due to higher oxidative stress under protein restricted diet, and the stress was partially removed when antioxidant vitamins were supplemented to the diet as previously described by Kim and Lee BM, (2001). In the present results suggested that curcumin protected against the lung toxicity induced by nicotine Treatment in rats.

XANTHINE OXIDASE (XOD): Xanthine oxidase is a superoxide-producing enzyme found normally in serum and the lungs, and its activity is increased during influenza A infection. During severe liver damage, xanthine oxidase is released into the blood, so a blood assay for XO is a way to determine if liver damage has happened. (Battelli, Maria Giulia *et al*., 2001). Because xanthine oxidase is a metabolic pathway for uric acid formation, the xanthine oxidase inhibitor allopurinol is used in the treatment of gout. Since xanthine oxidase is involved in the metabolism of 6-mercaptopurine, caution should be taken before administering allopurinol and 6-mercaptopurine, or its prodrug azathioprine, in conjunction. Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in blood and can cause health problems such as renal failure. There is no specific treatment, sufferers are advised by doctors to avoid foods high in purine and to maintain a high fluid intake.

Type I xanthinuria has been traced directly to mutations of the *XDH* gene which mediates xanthine oxidase activity. Type II xanthinuria may result from a failure of the mechanism which inserts sulfur into the active sites of xanthine oxidase and aldehyde oxidase, a related enzyme with some overlapping activities (such as conversion of allopurinol to oxypurinol). Inhibition of xanthine oxidase has been proposed as a mechanism for improving cardiovascular health. (Dawson J, Walters M 2006) A study found that patients with chronic obstructive pulmonary disease (COPD) had a decrease in oxidative stress, including glutathione oxidation and lipid peroxidation, when xanthine oxidase was inhibited using allopurinol. (Heunks LM, 1999) Oxidative stress can be caused by hydroxyl free radicals and hydrogen peroxide, both of which are byproducts of XO activity. (Higgins P, Dawson J, Walters M 2009). In the present study a significantly

decreased (-23.85%) activities of XOD were observed in the lung tissue of Nicotine treated (Nt) rats when compared to normal rats. In all the experimental animals significantly upregulated was observed (Nt+Cur Extract) by (-19.36%) activity of enzyme was observed in the lung tissue, (Table 3).

Nicotine, the major component of cigarette smoke, plays an important role in the development of lung complications. Earlystage disease can be treated with curative intent although the risk for relapse is notoriously high. Unfortunately, the majority of lung cancer patients present at an advanced stage. Despite an initial response to treatment, most of these late stage patients will eventually progress on standard therapy and die from their disease. Despite the complex nature of lung cancer biology, its molecular underpinnings are becoming increasingly clear (Salgia R, 2011 and Teixeira V, 2013).

Table 3. Changes in Xanthion oxidase (XOD) activity due to Nicotine treatment (Nt), and interaction of the both Nicotine+Curcumin for a period of 2 months over the control in Lung tissue of male albino rats. Values are expressed in μ moles of formazan formed / mg protein/hour

Name of the tissue	Control	Nicotine (Nt)	Nicotine+Curcumin
Lung tissue	85.45	65.07**	68.9*
	±4.99	±6.16	±6.98
		(-23.85)	(-19.36)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Curcumin is a potent "scavenger" of the superoxide radical, a free radical that initiates potentially harmful oxidative processes (Sreejayan N, Rao MN1996). Through in Curcumin also increases survival of cells exposed in vitro to the enzyme hypoxanthine/xanthine oxidase, which stimulates superoxide and hydrogen peroxide production. Curcumin demonstrates several other in vitro effects linked to free radical scavenging. Moreover, curcumin has also been shown to quench reactive oxygen species and scavenge superoxide anion radicals and hydroxyl radicals and strongly inhibits nitric oxide (NO) production by down-regulating inducible nitric oxide synthase gene expression (Ghoneim AI, 2009 and Wang WZ, 2008). Different metabolic states (hypoxia, ischemia) lead to the conversion of the dehydrogenase form of xanthine oxidoreductase to an oxidase form (XOD), which relates the metabolism of oxypurines with the generation of reactive oxygen radicals, conversion of dehydrogenase form of xanthine oxidoreductase to an oxidase form in hypoxic condition and generation of reactive oxygen radicals. This decreased in enzyme activity, most probably reflects the increased oxidative stress through the nicotine toxicity by producing the free radicals. The increase was observed in the red grape extract and leaf extract administrated rats in the lung tissue. In the combination treatment (Nicotine+Curcumin) slightly decreased was observed.

Glucose-6-Phosphate Dehydrogenase (G-6-PDH): G-6-PDH, the first and rate limiting enzyme of the pentose phosphate pathway, has long been regarded as important in the biosynthesis of sugar moiety of nucleic acids and determines the amount of NADPH by controlling the metabolism of glucose via pentose phosphate pathway. It has been traditionally thought that G-6-PDH was a typical "house keeping" enzyme that was regulated slowly by the ratio of NADPH and NADP (Kletzien *et al.,* 1994; Tian *et al.,* 1999). The production of NADPH required for the regeneration of glutathione in the mitochondria is critical for scavenging the mitochondrial ROS through glutathione reductase and glutathione peroxidase systems (Jo *et al*., 2001). G-6-PDH may have directly reduced the basic ROS formation and as a consequence, increased the cellular concentration of glutathione (Salvemini *et al.,* 1999). G-6-PDH plays a critical role in cell growth by providing NADPH for redox regulation (Tian *et al.,* 1998). In the present study the decrease (-42.78%) activities of G-6-PDH were observed in the lung tissue of Nicotine treated (Nt) rats when compared to normal rats. In all the experimental animals significantly upregulated was observed (Nt+Cur Extract by (-23.32%) activity of enzyme was observed in the lung tissue, (Table.4).

Table 4. Changes in Glucose-6-phosphate dehydrogenase activity due to Nicotine treatment (Nt) , and interaction of the both Nicotine+Curcumin for a period of 2 months over the control in Lung tissue of male albino rats. Values are expressed as μ moles of formazan formed/mg protein/hour

Name of the tissue	Control	Nicotine (Nt)	Nicotine+Curcumin
Lung tissue	82.66	47.29**	63.38*
	±8.20	±5.27	±8.99
		(-42.78)	(-23.32)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at $P < 0.05$
** Values are significant at $P < 0.01$
@ Values are non significant.

Nicotine induced oxidative stress conditions the activity of G-6-PDH was decreased in the lung tissues of rats in both age groups. Nicotine induced stress condition is linked to the metabolism of nicotine in the lung tissues. In the similar studies, Chennaiah *et al*., (2011) reported the G-6-PDH activity was decreased in all the skeletal muscle fibres with the administration of nicotine. Gumustekin *et al*., (2005) reported that administration of nicotine inhibited the G-6-PDH in rat tissues. Similar decrease in G-6-PDH activity in rat during various induced toxic stress conditions in different tissues was also reported (Cartana *et al.,* 1989; Pugazhenthi *et al.,* 1991). A decrease in G-6-PDH activity was observed in nicotine injected rats. Both doses of curcumin showed significantly higher G6PDH activity than that nicotine treated rats with no statistical significance from that of control. Treatment of nicotine group with both doses of curcumin almost restored G6PDH activity to normal level, with no dose dependent effect. To explain induced oxidative stress in rat lung tissue by scavenging various free radicals and increasing activities of antioxidative enzyme, such as glutathione -6-phosphate dehydrogenase (G6PDH) (Watanabe *et al*., 2000). The present study was designed to investigate the role of curcumin in reducing induced oxidative damage on lung tissue and in inducing antioxidants like G-6-PDH. G6PDH is the predominant source for cellular NADPH defense against oxidative stress (Tian WN *et al.,* 1999).

Gluthathione -S-Transferase (GST)

Glutathione s-transferase (GST; EC 2.5.1.18) isoenzymes are ubiquitously distributed in nature, being found in organisms as diverse as microbes, insects,plants,fish,birds and mammals (Hayes and Pulford 1995). The transferase possess various activities and participate in several different types of reaction.

Most of these enzymes can catalyse the conjugation of reduced glutathione (GSH) with compounds that contain an electrophilic centre through the formation of a thioether bond between the sulphur atom of GSH and the substrate (Chasseaud 1979). In addition to conjugation reactions, a number of GST isoenzymes exhibit other GSH-dependent catalytic activities including the reduction of organic hydro peroxides (ketter *et al*., 1990) and isomerisation of various unsaturated compounds (Benson *et al*., 1977; Jakoby and Habig 1980). These enzymes also have several non catalytic functions that ralate to the sequestering of carcinogens, intracellular transport of a wide spectrum of hydrophobic ligands, and modulation of signal transduction pathways (Cho *et al*., 2001). GST represent a complex grouping of proteins. Two entirely distinct superfamilies of enzyme have evolved that possess transferase activity (Hayes and Strange 2000). The first enzyme to be characterized were the cytosolic,or soluble,GSTs (Bolyland and chasseaud 1969).

Table 5. Changes in Glutathione-S-transferase activity due to Nicotine treatment (Nt), and interaction of the both Nicotine+Curcumin for a period of 2 months over the control in Lung tissue of male albino rats. Values are expressed as moles of formazan formed/mg protein/hour

Name of the tissue	Control	Nicotine (Nt)	Nicotine+Curcumin
Lung tissue	174.24 ±6.78	152.37** ±8.33 (-12.55)	160.23* ±7.42 (-8.04)

All the values are ± SD of six individual observations.
Values in parentheses denote per cent change over respective control.
* Values are significant at P < 0.05
** Values are significant at P < 0.01
@ Values are non significant.

In the present study the decrease (-12.55%) activities of Glutathione s-transferase were observed in the lung tissue of Nicotine treated (Nt) rats when compared to normal rats. In all the experimental animals slightly upregulated was observed (Nt+Cur Extract by (-8.04%) activity of enzyme was observed in the lung tissue, (Table-5). Kuttan *et al* (1985) examined the anticancer potential of curcumin in vivo in mice using Dalton's lymphoma cells grown as as cites. Initial experiments indicated that curcumin reduced the development of animal tumors. The initiation of gluthathione S-transferase (GST) activity (Kuttan *et al*., 1985; Sharma *et al*., 2001). In curcumin, the phenolic and the methoxy group on the phenyl ring and the 1,3-diketone system seem to be important structural features that can contribute to these effects. Another fact proposed in the literature is that the antioxidant activity increases when the phenolic group with a methoxy group is at the ortho position (Motterlini *et al*., 2000). Efficacy of curcumin appears to be related to induction of glutathione S-transferase enzymes (Sharma *et al*., 2004). Curcumin pre & post treatment in rats having isoprenaline induced pulmonary function.

Glutathione-S-transferase (GST) mainly detoxifies electrophilic compounds (Hemachand *et al*., 2002), and has a well-established role in protecting cells from mutagens and carcinogens as a free radical scavenger along with glutathione. In the present study, the decreasing level of GSH and decreased activity of GSH-dependent enzymes, i.e. GPx, GR, and GST may be due to increased utilization to scavenge the free radical generation. A decrease in the level of antioxidant status can lead to the excessive availability of super oxide and peroxyl radicals which in turn generate hydroxyl radicals, the results of this study show that Curcumin extract administration may generally restore the Glutathione-S-transferase activity inactivated due to nicotine administration in lung tissue. According to Biswas *et al*., (2010). Curcumin also exhibited protective action against the depletion of antioxidants like glutathione S-transferase (GST).

Conclusion

In conclusion the present investigation suggests that 2 months curcumin extract treatment significantly enhanced the antioxidant status in the lung of nicotine-treated rats. Previous studies have reported that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by a chemical carcinogen. However, it is suggested that curcumin might be a useful pulmonary protective agent in cancer therapy along with standard chemotherapeutic drug.The multiple beneficial effects make curcumin a promising dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

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