Vol 4 Issue 5 Feb 2015

ISSN No : 2249-894X

Monthly Multidisciplinary Research Journal

Review Of Research Journal

Welcome to Review Of Research

RNI MAHMUL/2011/38595 **ISSN No.2249-894X**

Review Of Research Journal is a multidisciplinary research journal, published monthly in English, Hindi & Marathi Language. All research papers submitted to the journal will be double - blind peer reviewed referred by members of the editorial Board readers will include investigator in universities, research institutes government and industry with research interest in the general subjects.

Address:-Ashok Yakkaldevi 258/34, Raviwar Peth, Solapur - 413 005 Maharashtra, India
Cell : 9595 359 435, Ph No: 02172372010 Email: ayisrj@yahoo.in Website: www.ror.isrj.org

Review Of Research
ISSN:-2249-894X
Impact Factor : 3.1402(UIF)
Vol. 4 | Issue. 5 | Feb. 2015
Available online at www.ror.isrj.org

PROTECTIVE EFFECT OF CURCUMIN ON NICOTINE-INDUCED TOXICITY OF LIVER AND KIDNEY IN RATS

[1]Fatma G. Metwally , [1]Sawsan H. Karam , [2]Elham M. Elshazli , [3]Amany Abdel Ghaffar, [3]Atef Mohamed Mahmoud and [3]Margret A. Aziz

[1]Histology, [2]Pathology and [3]biochemistry Departments, Ophthalmology Institute, Giza, Egypt.

Abstract:-The present study was carried out to determine the histopathological effects of nicotine, one of the most significant components of tobacco, on liver and kidney of rat and ameliorative effect of curcumin on liver and kidney damage. Thirty adult albino rats were divided into three groups; control group, nicotine group and nicotine + curcumin group. The rats in nicotine and nicotine + curcumin groups were injected intraperitoneally with 2.5mg/Kg of nicotine every day for eight weeks. The rats in nicotine+ curcumin group were additionally given 80mg/Kg of curcumin by stomach tube after 10 minutes from nicotine injection for eight weeks. Five animals from each group were killed after six weeks while the rest were killed after eight weeks. Biochemical results showing increased Malondialdehyde (MA) and nitric oxide (NO) and decreased reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (Ca) in nicotine treated groups. While in nicotine + curcumin groups showing improvement of the previous results. The liver and kidney were removed for histopathological processing and examined by light microscopy. The histopathological results revealed time-dependent degeneration in the liver and kidney in nicotine group. Regenerative changes in nicotine + curcumin group were observed when compared with nicotine group, but the improvement was better after six weeks than eight weeks.

Keywords:curcumin, nicotine-induced toxicity, liver, kidney, rats.

INTRODUCTION

Nicotine, a major toxic component of cigarette smoking, has been reported to induce oxidative stress both in vivo and in vitro (Suleyman *et al.*, 2000). Nicotine, found in tobacco, is a natural alkaloid and an agent that weakens the immune system. Nicotine and metabolites increase lipid peroxidation and also affect the activities of antioxidant enzymes, thus, causing oxidative damage (Siktar, 2011). It is also known that the level of oxidative damage in tissues causes pathologic conditions (Guan *et al.*, 2003; Yildiz, 2004 and Jensen *et al.*, 2012).

Medicinal plants and their active principles have received great attention as potential antiperoxidative agent (Lee and Park, 2003). Curcumin, an important constituent of turmeric (*Curcuma ionga* L.), has been widely used for centuries as an indigenous medicine (Dinkova-Kostova, 2002). Curcumin exhibits a wide range of pharmalogical effects such as antioxidant, antitumor, anti-inflammatory and hepatoprotective activities (Ammon and Wahl, 1991). Previous studies have shown the protective effects of curcumin against 1,2-dimethylhydrazine-induced colon cancer, and alcohol as well as carbon tetrachloride- induced hepatotoxicity (Akila *et al*., 1998; Devasena *et al.*, 200. and Rukkumani *et al*., 2002).

The objective of this study is to observe liver and kidney damage due to nicotine injection by means of light microscopy and to assess the protective of curcumin against this exposure.

MATERIALS AND METHODS

Animals:

Male albino rats (Wister strain), with body weight range of 120-140g were obtained from the Central Animal House, Research Institute of Ophthalmology, Giza, Egypt. The animals were housed to polypropylene cages and provided with food and water ad libitum. The animals were maintained under standard conditions of temperature at 25°C

Chemicals:

Nicotine (nicotine ditartarate dehydrate) was purchased from Sigma Chemical Company, St Louis, Mo, USA and dissolved in distilled water . Curcumin (65%-70% purity) was purchased from Sigma Chemical Company.
Experimental Design:

Thirty albino rats were divided into three equal groups; control group, nicotine group and nicotine + curcumin group. The rats in control group were not given any treatment. The rats in nicotine and nicotine + curcumin groups were injected intraperitoneally with 2.5 mg/kg of nicotine every day for eight weeks. The rats in nicotine + curcumin group were additionally given 80 mg/kg of curcumin by stomach tube at the same time for eight weeks. Five animals from each group were killed after six weeks while the rest were killed after eight weeks.

Biochemical Analysis:

Blood was drawn from each rat, serum was separated, and a hemolysate was prepared and samples were stored at -70°C until analysis.

Analysis of antioxidant enzymes:

Catalase (CAT) activity was determined by the method of Sinha (1972). In this test, dichromatic acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H_2O_2), with the formation of perchloric acid as an unstable intermediate.

Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974). In this test, the degree of inhibition of pyrogallol auto-oxidation by the supernatant of the hemolysate was measured.

Estimation of reduced glutathione:

Reduced glutathione (GSH) was determined according to the method of Ellman (1959).

Estimation of nitric oxide:

Nitric oxide was estimated by using the Griess method (Lepovire *et al.*, 1990).

Estimation of malondialdehyde:

The extent of lipid peroxidation was determined by the method of (Sener *et al.*, 2007).

Statistical analysis

Data from biochemical investigation were analyzed using analysis of variance (ANOVA). The results were considered statistically significant if the $p < 0.05$.

Histopathological Examination:

At the end of each experiment, the rats were killed by cervical dislocation. The livers and kidneys were removed and rapidly fixed in 10% formalin for histopathological processing. After fixation each liver and kidney tissues were routinely processed and embedded in paraffin. After embedding, 5µm thick sections were taken from tissue blocks. All sections of livers and kidneys were stained with haematoxylin and eosin and examined by light microscopy.

RESULTS

Biochemical Results:

Table I: Activities of SOD, CAT and the levels of reduced glutathione, malondialdehyde and nitric oxide in blood of experimental groups.

Parameters	SOD (U/g Hb)	CAT (U/g Hb)	GSH (mg/dl eryth)	MDA (nmol/ml)	NO (µmol/L)
Control	2494 ± 79	128.4±2.5	60.2±3.6	3.47±0.19	36.38±7.15
Nicotine After 6 weeks	1895±132[a]	96.3±1.5[a]	41.6±5.2 [a]	6.98±0.08 [a]	51.5±3.5 [a]
Nicotine After 8weeks	1471±150[ab]	86.2±1.22[ab]	32.9±5.2 [ab]	5.99±0.15 [a]	50.90±8.53 [a]
Nicotine + curcumin After 6weeks	2044.4±180[abc]	120±8.2[bc]	54.1±1.3 [abc]	4.12±0.09 [bc]	40.78±3.4 [bc]
Nicotine + curcumin After 8weeks	2389±59[bc]	125±5.3[bc]	58.2±2.2 [bc]	4.08±0.05 [bc]	37.56±2.1 [bc]

Values are mean ± SD; n=6.

a, Significant difference ($p<0.05$) when compared with 'control' group rats.
b, Significant difference ($p<0.05$) when compared with group II (rats treated with nicotine after 6 weeks). c, Significant difference ($p<0.05$) when compared with group III (rats treated with nicotine after 8 weeks).

Activities of SOD and CAT and the levels of GSH, MDA and NO in the hemolysate of control and experimental animals in each group are shown in Table (1). The activities of SOD and catalase were significantly decreased in nicotine-treated rats when compared to control. Oral administration of curcumin to nicotine-treated rats significantly increased the activity of SOD and catalase when compared with animals treated with nicotine alone. The levels of MDA and NO were significantly elevated in animals treated with nicotine when compared to controls. Administration of curcumin to nicotine-treated animals significantly decreased the level of MDA and NO when compared with nicotine treated animals. On the other hand, the level of reduced glutathione was decreased in the hemolysate of nicotine-treated rats as compared with control. Supplementation of curcumin to nicotine-treated rats significantly elevated the levels of GSH.

Histopathological Results:

Liver

Light microscopic examination of control liver sections was shown to be formed of well classical hepatic lobules. The lobule appeared hexagonal in shape with central vein forming its central axis and portal tracts present at the angles of the hexagon. The hepatocytes appeared polyhedral in shape with acidophilic cytoplasm and rounded vesicular nuclei. They were arranged in anastomsing cords usually one or two cells thick that radiate from the central vein toward the portal tract. Between these cords, the blood sinusoids were seen lined with flattened endothelial cells with deeply stained nuclei and kupffer cells with rounded nuclei (Fig. 1).

Animals treated with nicotine for six weeks showed vacuolated hepatocytes in the peripheral regions with pale stained cytoplasm (Fig. 2). Central veins appeared dilated and congested with cellular infiltration around as well as focal necrotic area was observed. The nuclei of some hepatocytes were fragmented or pyknotic (Fig. 3).The examination of liver of rats that received nicotine + curcumin for six weeks appeared more or less normal except slightly dilated blood sinusoid (Fig. 4).

After eight weeks of nicotine injection, loss of architecture was observed. Severe dilatation of central veins appeared with mononuclear cell infiltration as well as extravasations of blood elements (Fig. 5).
Less regenerative changes were observed in the liver of rats that received nicotine + curcumin for eight weeks (Fig. 6).

Kidney

Light microscopic examination of control kidney showed normal appearance of glomerulus with its capillary tuft surrounded by capsular space and Bowman's capsule. The proximal and distal convoluted renal tubules also appeared normal (Fig. 7). Animals treated with nicotine for six weeks showed shrinkage of some glomeruli with wide capsular space. Some tubules appeared dilated with swelling of the epithelial lining and prominent nuclei, some being almost solid eosinophilic hyaline material (Fig. 8). Giving the animal curcumin for the same period, improvement was clear as the glomeruli appeared less affected (Fig. 9), tubules appeared normal except for the presence of few red blood cells in the interlobular spaces (Fig. 10). Light microscopic examination of treated kidneys with nicotine for eight weeks showed variable degrees of glomeruli degeneration, many appeared shrunken with wide capsular space; others appeared necrotic leaving glomeruli debris in the capsular space (Fig. 11). Many tubules appeared dilated, some of which contained red blood cells. The epithelial lining was flattened, some showed loss of cell lining, vacuolation of the cellular lining and hyalinization of others, tubular epithelial lining appeared acellular in some foci (Fig. 12). Addition of curcumin for eight weeks showed less improvement as glomeruli appeared slightly shrunken with widening capsular space .The tubules appeared less affected, few of which appeared dilated with red blood cells (Fig. 13).

DISCUSSION

Enhanced lipid peroxidation associated with depletion of antioxidants in the liver, lung and kidney is a characteristic observation in nicotine- treated rats (Siktar, 2011 and Kalpana and Menon, 2004). Nicotine, a major toxic component of cigarette smoking, has been recognized to result in oxidative stress by inducing the generation of free radicals and reactive oxygen species (Ekinci *et al.*, 2010). As a consequence, these radicals interact with cell components such as lipids, proteins, DNA, RNA, carbohydrates and enzymes (Ekinci *et al*., 2010 and Ekinci, 2011). The interaction usually causes a decrease or even loss of function of these molecules.

The liver seemed to be the appropriate organ to give a true reflection to the extent of the toxicity caused by nicotine injection. In the present study, nicotine injection resulted in degeneration of hepatocytes, expanded portal tracts with infiltrating inflammatory cells and dilatation of central veins. These changes were observed at the periphery of the hepatic lobules then extended to involve the whole lobule. This obvious hepatic toxicity following nicotine injection, was previously reported (Czekej *et al*., 2002; Ghaly *et al*., 2002; Balakrishnan and Menon 2007; Iranloye and Bolarinwa 2009; El-Sokkary et al., 2007 and Mercan and Eren, 2013). The progressive dilatation of the vessels in the liver could be considered as a reaction change that might be related to increased level of prostaglandin (PG) synthesis where these PGs induced smooth relaxation and consequent vasodilatation either directly or through releasing other vasodilator substances in blood (Backhle *et al.*, 1979). The presence of infiltrating inflammatory cells, might be explained as a defense reaction of the lobule in response to the toxicity of injected nicotine (Gorrod and Jenner, 1975). The efficiency of the hepatocytes to perform their metabolic functions is dependent on the incoming blood, which is influenced by the availability of oxygen and nutrients. This in turn is affected by the presence of toxic substances in the blood that bathes the hepatocytes.

In the present study, vacuolation and necrosis were more obvious in the peripheral hepatocytes. The same findings were explained by Willkeins *et al*. (1990) who stated that the blood circulates in the peripheral sinusoids before others. Thus the peripheral cells become exposed to higher concentration of toxin more than the central one and so marked lesions were produced in the peripheral hepatocytes. As regard to the kidney, Light microscopic evaluation of the kidney from the rats injected with nicotine revealed shrunken glomeruli, wide capsular space, swelling of tubular epithelial lining , some of these cells being solid eosinophilic and hyalinized. Red blood cells were obvious in all sections examined between the tubules. Similar changes were detected by Pekmez *et al.* (2010) as atrophic renal corpuscles and tubular degeneration were observed. These changes may be because the nicotine –induced free radicals react with biomembranes causing oxidative destruction of polyunsaturated fatty acids and forming cytotoxic aldehydes by lipid peroxidation (Yildiz *et al*., 1999). Lipid peroxidation can be used as an index for measuring the extent of damage that occurs in membranes of tissues as a result of free radicals generation (Jason et al., 1998).

Antioxidants are capable of inhibiting the oxidation of several molecules. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radicals intermediates and inhibit other oxidation reactions (Reiter, 1995; Helen et al., 2000 and Helen *et al*., 2003). In many recent studies, antioxidants are proved to ameliorate changes in tissues caused by cigarette smoking or nicotine injection (Mercan and Eren 2013; Iranloye and Bolarinwa, 2009; Ekinci *et al*., 2010; Backhle *et al.*, 1979; Yildiz *et al.*, 1999 and El-Sokkary *et al*., 2006).

Administration of curcumin reversed the changes by nicotine in this study. Also, no red blood cells could be detected in between the tubules in the kidney as well as no extravasation of blood cells in the liver. These support the hypothesis that plant products are effective antioxidative agents. Curcumin by scavenging or neutralizing free

radicals, interacting with oxidative cascade, quenching oxygen, inhibiting oxidative enzymes like cytochrome P450, and by chelating metal ions like Fe2+, inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function (Balasubramanyam *et al.*, 2003 and Pulla and Lokesh 1994). Thus, curcumin may stabilize the cell membrane and significantly reduce the extent of lipid peroxidation in the liver and kidney (Kalpana and Menon, 2004).

Curcumin significantly enhanced the antioxidant status in the liver, lung and kidney of nicotine-treated rats (Rukkumani *et al*., 2002). Previous studied study has reported that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by a chemical carcinogen (Singletary *et al*., 1998.). Curcumin has been reported to protect hepatocytes against alcohol and polyunsaturated fatty acid (PUFA)-induced liver toxicity (Rukkumani *et al*., 2002). Having polyphenolic structure and β-diketone functional groups, curcumin is a stronger antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group (Phan et al., 2001).

These observations are consistent with those of Kalpana and Menon (2004) who proved that administration of curcumin and curcumin analog significantly lowered the lipid peroxidation and enhanced the antioxidant status. They suggest that curcumin and curcumin analog exert their protective effects by modulating the extent of lipid peroxidation and enhancing the antioxidant status.

Rukkumani and his collogue (2003) stated that effective antioxidant property of curcumin decreases the utilization of vitamins C&E in the liver and thus maintains their levels. Thus curcumin exerts its protective effect against nicotine-induced toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system. The results of the present study assure that curcumin can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

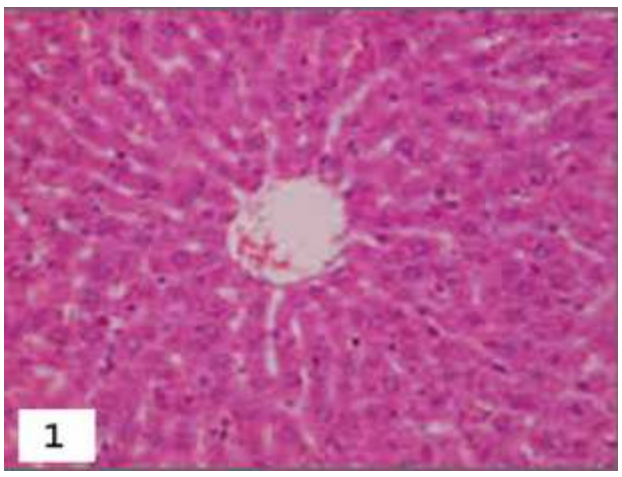

Fig. 1: Light micrograph of control rat liver tissue showing classical hepatic lobules. The hepatocytes appeared polyhedral in shape with acidophilic cytoplasm and rounded vesicular nuclei. The blood sinusoids were seen lined with flattened endothelial cells with deeply stained nuclei (H&EX250).

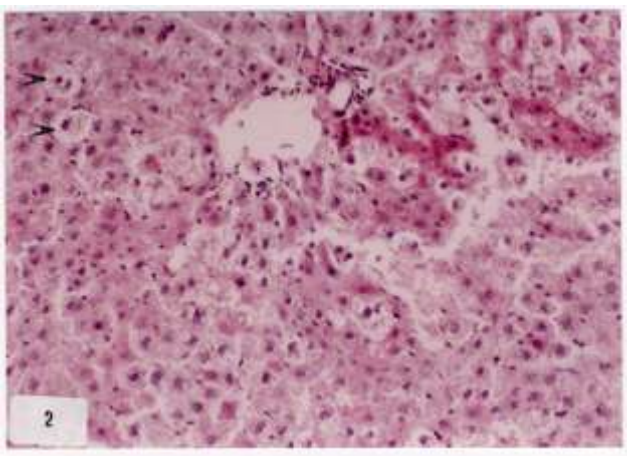

Fig. 2: Light micrograph of rat liver tissue subjected to nicotine for six weeks showing vacuolated hepatocytes in the peripheral regions with pale stained cytoplasm (H&EX 250).

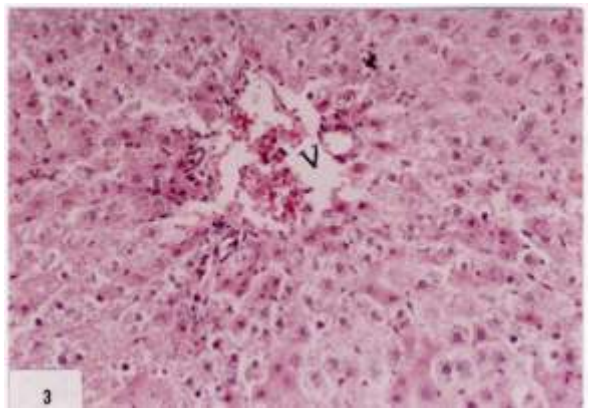

Fig. 3: Light micrograph of rat liver tissue of another field from the second group showing dilated and congested central vein (V) with cellular infiltration around as well as focal necrotic area (H&EX250).

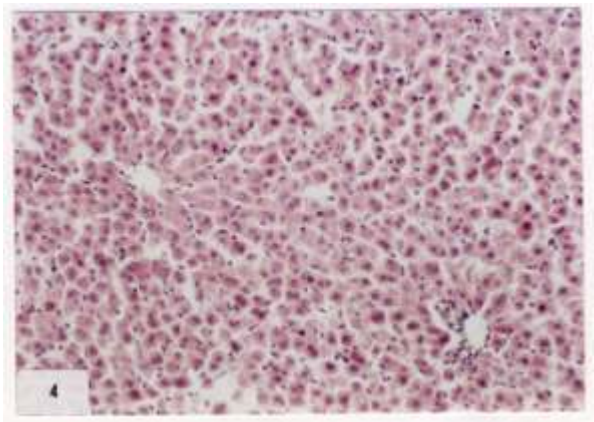

Fig. 4: Light micrograph of rat liver tissue subjected to nicotine and curcumin for six weeks the liver appeared more or less normal (H&EX250).

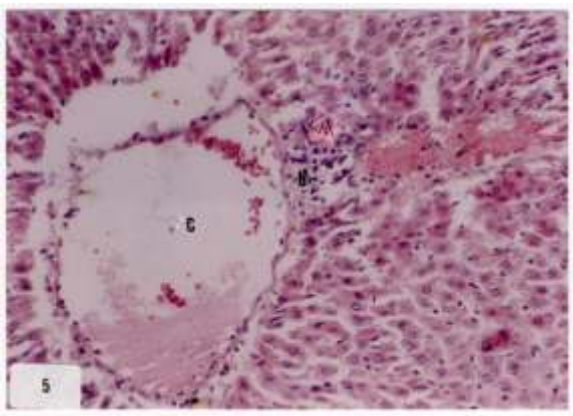

Fig. 5: Light micrograph of rat liver tissue subjected to nicotine for eight weeks showing severe dilatation of central vein(c) with infiltration of mononuclear cells (M) as well as extravasations of blood elements (H&E250).

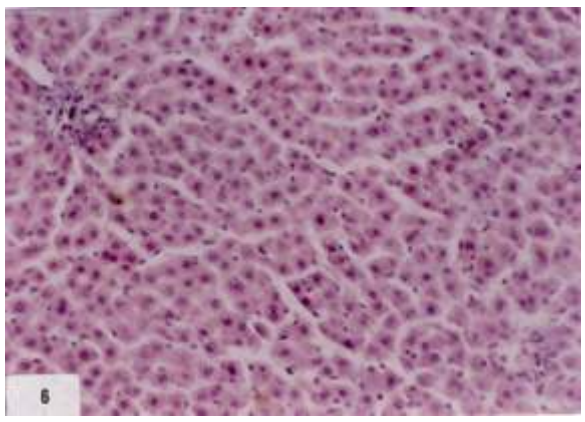

Fig. 6: Light micrograph of rat liver tissue subjected to nicotine and curcumin for eight weeks showing improvement of liver tissue (H&EX250).

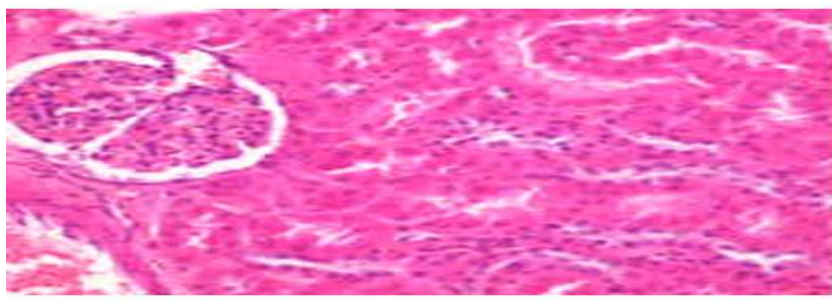

Fig. 7: Light micrograph of control rat kidney tissue showing normal appearance of glomerulus with its capillary tuft surrounded by capsular space and bowman's capsules. Renal tubules also appeared normal (H&E x250).

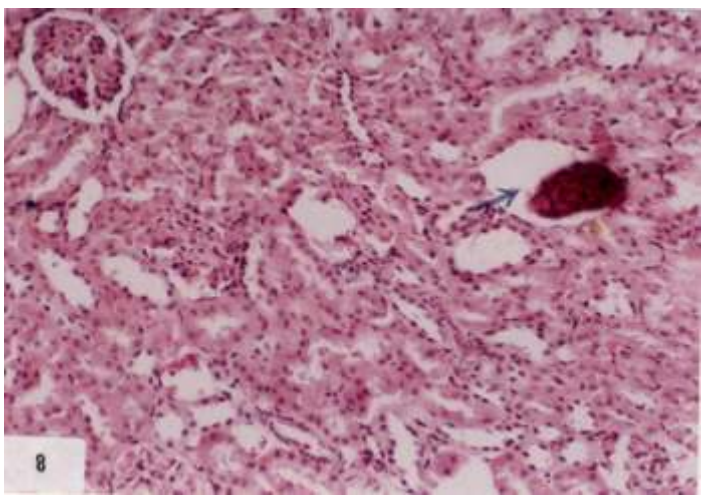

Fig. 8: Light micrograph of rat kidney tissue subjected to nicotine for six weeks showing shrinkage of some glomeruli with wide capsular space, other glomeruli appear necrotic(arrow) .The epithelial lining of the tubules showing swelling and prominent nuclei (H&E x 250).

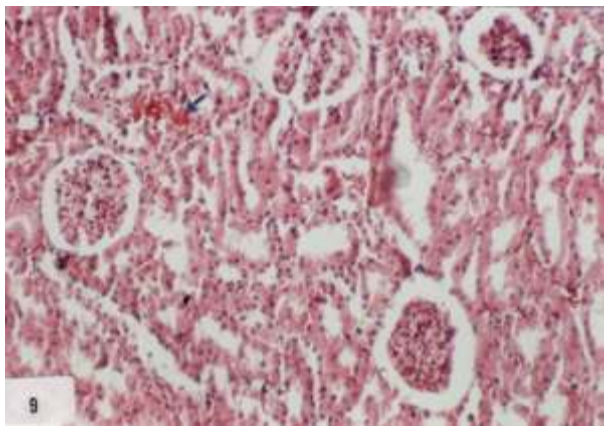

Fig. 9: Light micrograph of rat kidney tissue subjected to nicotine and curcumin for six weeks showing normal glomeruli with interlobular red blood cells (arrow) (H&E x250).

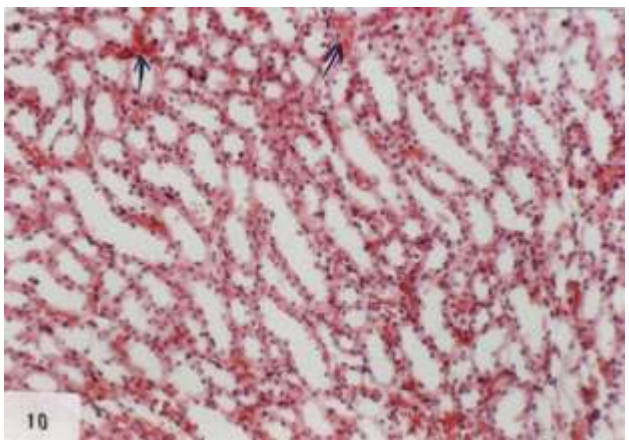

Fig. 10: Light micrograph of rat kidney tissue subjected to nicotine and curcumin for six weeks showing more or less normal tubules with few interlobular red blood cells (arrows) (H&E x 500).

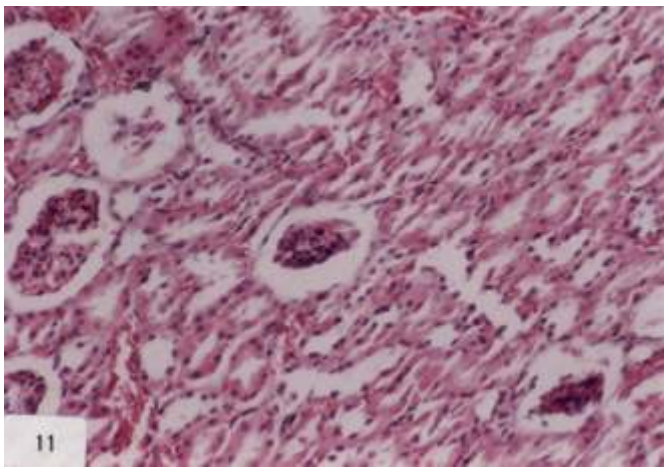

Fig. 11: Light micrograph of rat kidney tissue subjected to nicotine for eight weeks showing shrinkage of some glomeruli and necrotic of others (H&E x 250).

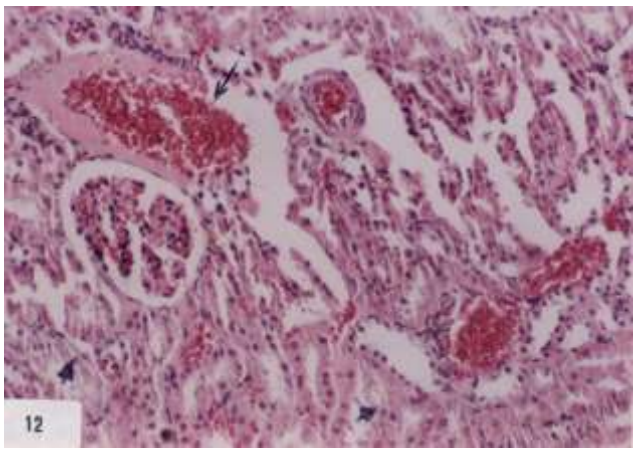

Fig. 12: Light micrograph of rat kidney tissue subjected to nicotine for eight showing many tubules appeared dilated, some of which contained containing hyaline casts red blood cells (arrow). Tubular epithelial lining appeared a cellular in some foci (arrow head) (H&E x 250).

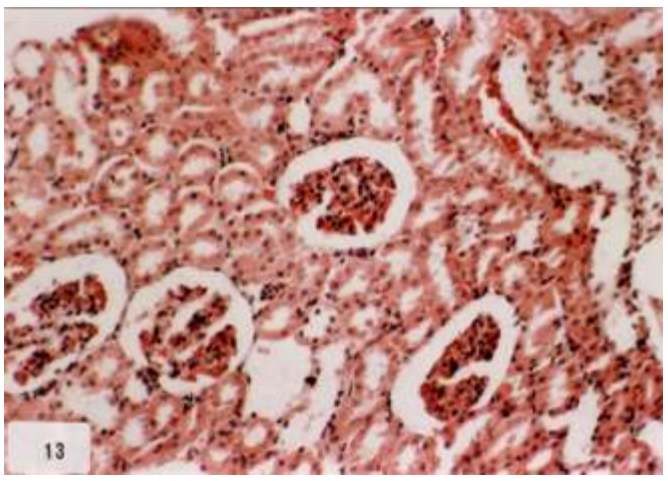

Fig. 13: Light micrograph of rat kidney tissue subjected to nicotine and curcumin for eight weeks showing slightly shrinkage of some glomeruli with wide capsular spaces. Note, absence of inter and intralobular red blood cells (H&E x250).

REFERENCES

1.Akila, G., Rajakrishnam V, Viswanathan P, Rajasekaran KN and Menon VP 1998. Effects of curcumin on lipid profile and lipid peroxidation status in experimental hepatic fibrosis. Hepatol. Res., 11: 147-157.
2.Ammon, H.P.T. and Wahl, M.A. 1991. Pharmacology of Curcma ionga. Planta Med., 57: 1-7.
3.Backhle, YS, Hartiala J, Toivonan H, and Votilla P 1979. Effects of cigarette smoke on the metabolism of vasoactive hormones in the isolated lung. BR. J. Pharmacol., 65: 495-499.
4.Balakrishnan, A, and Menon VP 2007. Protective effect of hesperidine on nicotine induced toxicity in rats. Indian J. Exp. Biol., 45: 194-202.
5.Balasubramanyam, M, Koteswari AA, Kumar RS, Monickaraj SF, Maheswari JU and Mohan, Y 2003. Curcumin–induced inhibition of cellular reactive oxygen species generation: novel therapeutic implications. J. Biosci., 28: 279-283.
6.Czekej, P, Palasz A, Lebada-Wybony T, Nowaczyk-Dura G, Karczewska W, Florek E, et al. 2002 Morphological changes in lungs, placenta, liver and kidneys of pregnant rats exposed to cigarette smoke. International Archives of Occupational and Environmental Health, 75: 27-35.
7.Devasena, T, Rajasekaran KN and Menon VP 2002. Bis-1,7-(2-hydrxyphenyl)-1,6-diene-3,5-dione(a curcumin analog) ameliorates DMH –induced hepatic oxidative stress during colon carcinogenesis. Pharmacol. Res., 46: 39-45.
8.Dinkova-Kostova, AT 2002. Protection against cancer by plant phenyl propenoids: induction of mammalian

anticarcinogenic enzymes. Mini. Rev. Med., 2: 595-610.
9.Ekinci, D, Ceyhun SB, Senturk M, Erdem D, Kufrevioglu OI and Supuran CT 2011. Characterization and anions inhibition studies of an α-carbonic anhydrase from the teleost fish Dicentrarchus Iabrx. Bioorganic and Medicinal Chemistry Letters 19(2): 744- 748.
10.Ekinci, D, Senturk M., Beydmir S, Kufrevioglu OI and Supuran CT 2010. An alternative purification method for human serum paraoxnase 1and its interactions with sulfonamides.Chemical Biology and Drug Design, 76(6): 552-558.
11.Ellman, GL 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys, 82: 70–77.
12.El-Sokkary, GH, Cuzzocrea S and Reiter RJ 2007. Effect of chronic nicotine administration on the rat lung and liver: Beneficial role of melatonin. Toxicology, (239): 60-67.
13.El-Sokkary, GH, HA, Khidr BM and Younes HA 2006. Role of melatonin in reducing hypoxia – induced oxidative stress and morphological changes in the liver of male mice. European J. pharmacology, 540: 107-114.
14.Ghaly, MA, Khedr EG and Abdel Aleem A 2002. A comparative study of nicotine effect on the liver of albino rat. Egyyp. J of Hosp. Med., 10: 130-144.
15.Gorrod, JW and Jenner P. 1975. The metabolism of tobacco alkaloids. In Essays in Toxicology, (6): 35-78, New York.
16.Guan, Z.Z., Yu WF, and Nordberg A 2003. Dual effects of nicotine on oxidative stress and neuroprotection in PC 12 cells. Neurochemistry International, 43: 243-249.
17.Helen, A, Krishnakumar K, Vijayammal PL, August KT 2003. A comparative study of antioxidants S-allyl cysteine sulfoxide and vitamin E on the damages induced by nicotine in rats. Pharmacology, 67: 113-117.
18.Helen, A., Krishnakumar K., Vijayammal PL, August KT 2000. Antioxidant effects of anion oil (Allium cepa linn) on the damages induced by nicotine in rats as compared to alpha –tocopherol. Toxicol. Lett., 116: 61-68.
19.Iranloye, BO and Bolarinwa AF 2009. Effect of nicotine administration on weight and histology of some vital visceral organs in female albino rats .Nigerian J of Physiological Science, 24(1): 7-12.
20.Jason, D.M. , Balz F , Atkinson W.L., Michael J.G. , Sean M.L., Yu S, William E S , John AO and Jackson R.L. 1998. Increase in Circulating Products of Lipid Peroxidation F2-Isoprostanes in smokers-Smoking as a cause of Oxidative Damage, The New England J. Med., 18: 332.
21.Jensen, K., Nizamutdinov D., Guerrier M., Afroze S., Dostal D and Glaser S. 2012. General mechanisms of nicotine-induced fibrogenesis, FASEB J. 26: 4778- 4787.
22.Kalpana, C, and Menon VP, 2004. Inhibition of nicotine-induced toxicity by curcumin and curcumin analog: A comparative study. J. Med. Food, 7(4): 467-471.
23.Kalpana, C, and Menon VP, 2004. Modulatory effects of curcumin on lipids peroxidation and antioxidant status during nicotine –induced toxicities, Pol. J. pharmcol., 56: 581-586.
24.Kalpana, C. and Menon VP. 2004. Protective effect of curcumin on circulatory lipid peroxidation and antioxidant status during nicotine –induced toxicity. Toxicol. Mech. Methods, 14: 339-343.
25.Lee, BM, and Park KK 2003. Beneficial and adverse effects of chemoprotective agents. Mutat. Res., 265-270.
26.Lepovire, M, Chenais B, Yapo A. et al. 1990. Alterations of ribonucleotide reductase activity following induction of nitrite-generating pathway in adenocarcinoma cells. J. Biol. Chem., 265: 14143-9.
27.Marklund, S. and Marklund G. 1974. Involvement of the Superoxide Anion Radical in the Autoxidation of Pyrogallol and a Convenient Assay for Superoxide Dismutase. Eur. J. Biochem., 47: 469-474.
28.Mercan, S and Eren B 2013. Protective role of melatonin supplementation against nicotine – induced liver damage in mouse. Toxicol. Ind. Health, 29(10): 888-896.
29.Pekmez, H, Ogeturk M, Ozyurt H, Sonmez MF, Colakoglu N and Kus I. 2010. Ameliorative effect of caffeic acid phenylethyl ester on histopathological and biochemical changes induced by cigarette smoke in rat kidney. Toxicology and Industrial Health, 26(3): 175-182.
30.Phan, TT, See ST, and Chan SY 2001. Protective effects of curcumin against oxidative damage on skin cells in vitro: its implications for wound healing. J. Trauma, 51: 927-283.
31.Pulla, RA and Lokesh BR 1994. Effect of dietary turmeric (Curcuma Ionga on iron-induced lipid peroxidation in the rat liver. Food Chem. Toxicol., 32: 279-283.
32.Reiter, RJ 1995. Oxidative process and antioxidative defense mechanisms in the aging brain .FASEB J., 9: 526-533.
33.Rukkumani, R, Balasubashini S, and Menon V.P. 2002. Comparative effects of curcumin and photo –irradiated curcumin on alcohol and polyunsaturated fatty acid – induced toxicity. Pharmacol. Res., 46. 257-264.
34.Rukkumani, R, Balasubashini S, and Menon V.P. 2003. Protective effects of curcumin and photoirradiation curcumin on circulatory lipids and lipid peroxidation products in alcohol and polyunsaturated fatty acid-induced toxicity. Phytother. Res., 17: 925-929.
35.Sener, G, Toklu HZ, and Cetinel S 2007. ß-Glucan protects against chronic nicotine induced damage in rat kidney

and bladder. Environmental Toxicology Pharmacology, 23: 25-32.
36.Siktar, E., Ekinci D., Beydemir S, Gulcin I. and Gunay M. 2011. Protective role of L-carnitine supplementation against exhaustive exercise induced oxidative stress in rats. European J of pharmacology, 668(3): 407-413.
37.Singletary, K., Mac Donald C., Lovinelli M., Fisher C. and Wallig M. 1998. Effect of the ß-diketoknes diferuloylmethane (curcumin) and dibenzoylmethane on rat mammory DNA adducts and tumor induced by 7,12-dimethylbenz(a) anthracane. Carcinogenesis, 19: 1039-1043.
38.Sinha, AK 1972. Colorimetric assay of catalase. Anal Biochem, 47: 389–394.
39.Suleyman H., Gumustekin K., Toysi S., Keles S., Oztasan N., Aktas O., Altinkaynak K et al. 2000. Beneficial effects of Hippophae rhamnoides L. on nicotine induced oxidative stress in rat blood compared with vitamin E. Biol. Pharm. Bull., 25: 1133-1136.
40.Willkens, RF, Leonard PA, Tolman KG, Ward JR, Marks CR, Greene ML, Roth GJ, Jackson CC, Cannon GW and Lee RG 1990. Drug hepatotoxicity. Cell. Biol. Toxicol., 49: 591.
41.Yildiz, D 2004. Nicotine, its metabolism and an overview of its biological effects. Toxicon., 43: 619-632.
42.Yildiz, D, Liu YS, Ercal N and Armstrong DW 1999. Comparison of pure nicotine and smokeless tobacco extract induced toxicities and oxidative stress. Archives Environ. Contam. Toxicol, 37: 434-439.