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RESEARCH ARTICLE

Histopathological Experimental Study for CurcuminToxic effect in Lab Animals

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Abstract

Curcumin - the bio active compounds present in spices-turmeric (Curcuma longa), a perennial herb of the family Zingiberaceae. This herb is a native of southern Asia and the East Indies; it is used principally as a constituent of curry powders and other condiments. The major compound, curcumin, provides yellow color in food.Even though this plant has been used for a very long time, there are only a few published articles on its toxicity, and adverse effects were not reported. Therefore,the present studies were conducted to determine the toxicity of turmeric powder in rabbits in order to obtain scientific information about its safety.Toxicity of turmeric powder at(250µg/ml per day) for two weeks was investigated by histopathological changes for (lung, liver, heart and intestine). Rabbits treated with turmeric powder had an organs with epithelial erosion, PMN infiltration referring to acute inflammation, Macrovesicular fatty change associated with metabolic disturbances and is generally readily reversible, whereas microvesicular fatty change is more likely a reflection of toxicity, as well as, increase in the percent of proliferating cells, increasing cells size and change the morphological feature of proliferating cells, where take abnormal shape, compared to the control animals.

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Introduction

Curcumin is a yellow–orange pigment obtained from the plant Curcuma longa. The powdered rhizome of this plant,called turmeric, is a common ingredient in curry powders and has a long history of use in traditional Asian medicine for a wide variety of disorders. In the last decade a large number of reports have been published on the beneficial effects of curcumin, and it has repeatedly been claimed that this natural product is efficient and safe for the prevention and treatment of several diseases including cancer (1–2). The fact that curcumin also undergoes extensive metabolism in intestine and liver (6,7) means that high concentrations of curcumin cannot be achieved and maintained in plasma and tissues after oral ingestion. This is a major obstacle for the clinical development of this agent and suggests that the therapeutic potential of oral curcumin is limited. But there is accumulating evidence that curcumin may not be so effective and safe.High number of reports suggests that curcumin may cause toxicity under specific conditions. Goodpasture and Arrighi found that turmeric caused a dose and time-dependent induction of chromosome aberrations in several mammalian cell lines; these alterations were observed at concentrations of 10 lg mL (23).Accumulating data have demonstrated since then that curcumin can induce DNA damage and chromosomal alterations both in vitro and invivo at concentrations similar to those reported to exert beneficial effect (24–25). For instance, curcumin concentrations of 2.5and 5 µg mL were shown to induce DNA damage to mitochondrial and nuclear genomes in cells (26).These reports raise concern about curcumin safety, as the induction of DNA alterations is a common event in carcinogenesis.The National Toxicology Program (USA) published an extensive report on the toxic and carcinogenic properties of an organic extract of turmeric, called turmeric oleoresin (27).This extract is commonly added to food items and contains apercentage of curcumin (79–85%) similar to that of

commercialgradecurcumin., The possible toxic and carcinogenic effects were evaluated in rats and mice fed diets containing several concentrations of turmeric oleoresin for 3 months and 2 years In the 2-year feeding studies, turmeric oleoresin ingestion was associated with increased incidences of ulcers, hyperplasia, and inflammation of the fore stomach, cecum and colon in male rats and of the cecum in female rats. In female mice, ingestion of diets containing turmeric oleoresin was associated with an increased incidence of thyroid gland follicular cellhyperplasia.The report also concluded that there was equivocal evidence of carcinogenic activity in female rats, female mice, and male mice. These conclusions were based on increased incidences of clitoral gland adenomas in female rats, hepatocellular adenomas in female mice, and carcinomas of the small intestine and hepatocellular adenomas in male mice. The increased incidence of carcinomas of the small intestine was observed in mice taking average daily doses of curcuminof 0.2 mg kg body weight (27). A recent report has also shown that curcumin can promote lung cancer in mice (28).These negative effects of curcumin may be mediated by several possible mechanisms. Evidence suggests that reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide may play an important role in carcinogenesis (29). This evidence is based on the facts that (i) ROS can induce cell malignant transformation, (30) (ii) cancer cells commonly have increased levels of ROS,41–43 and (iii) the malignant phenotype of cancer cells can be reversed by reducing the cellular levels of ROS (31). Experimental studies have demonstrated that, although low concentrations of curcumin induce antioxidant effects, higher concentrations of this compound increase the cellular levels of ROS (32–33) The presence of 2 a,b-unsaturated ketones in the chemical structure of curcumin may also mediate some of its negative properties. These chemical groups are known to react covalently with exposed thiol groups of cysteine residues of proteins through a reaction termed Michael addition.

This reactionmay explain, for instance, why curcumin generates ROS by irreversibly modifying the antioxidant enzyme thioredoxinreductase (34), why curcumin induces topoisomerase II-mediated DNA damage (35) and why curcumin inactivates the tumor suppressor protein p53 (36). Curcumin has also been shown to inhibit the activityof the drug-metabolizing enzymes cytochrome P450, glutathione-S-transferase (39). The inhibition of these enzymes in people taking curcumin may lead to an undesired increase in the plasma concentrations of some drugs and cause toxicity (40)

5- Material and Methods

- Preparation of curcumin samples

curcumin samples were obtained from Baghdad market, were prepared by dissolving (250mg) in 1L of D.W, the final concentration (250µg/ml).

- Experimental animals

Four young Iraq White rabbits (weight, 2.5 to 3.0 kg) were used in this experiment, they were fed stock ----, carrot, and ----as well as, water was supplied *ad libitum.*

- Animal treatments

Animals were divided equally into two groups, group A, orally treated with curcumin (three dosage at each day), while group B (negative control group without infection), received water and diet without curcumin.

- Histopathological and histochemical studies

All animals were anesthetized by intramuscular injection of xylazine (3 mg/kg of body weight) and ketamine hydrochloride (35 mg/kg), after that killing all groups. All organs collected from animals groups and then transported to glass containers containing (10%formaline), each container labeled with the number and give specific symbols for each sample.

After those samples were transported to thehistopathological lab for evaluate structural alterations in the tissues after treated with curacumin, all tissue sections stained by Haematoxylin and Eosin stain.

6- Results and Discussion

Results and Discussion

Current results, demonstrates a histological sections for (lung, heart, spleen, and intestine) in rabbits groups treated with curacumin at (250µg/ml per day) using (H&E, X200).Figure(3-1) showed pathologic changes in the intestine of treated rabbits include epithelial erosion, PMN infiltration referring to acute inflammation, as well as, increase in the

percent of proliferating cells, increasing cells size and change the morphological feature of proliferating cells, where take abnormal shape, furthermore, intestine section showed Macrovesicular fatty change, and enlarged villi with irregular brush border and take abnormal shapes. This phenomena which called Pautz-syndrome, its accrue in the intestine when the body exposed to carcinogenic material, where turameric plant contain cumarine material when orally intake degraded by juice and loss its advantage where converted to carcinogenic material, (42) referred to that the Macrovesicular fatty change associated with metabolic disturbances and is generally readily reversible, whereas microvesicular fatty change is more likely a reflection of toxicity

Only a minimum portion of the absorbed curcumin was traced in serosal fluid at the end of 3 h incubation period, while most of it was still present in intestinal tissue. The relatively lesser recovery of curcumin in its native form suggested transformation of this compound in the intestine to a certain extent during its absorption.

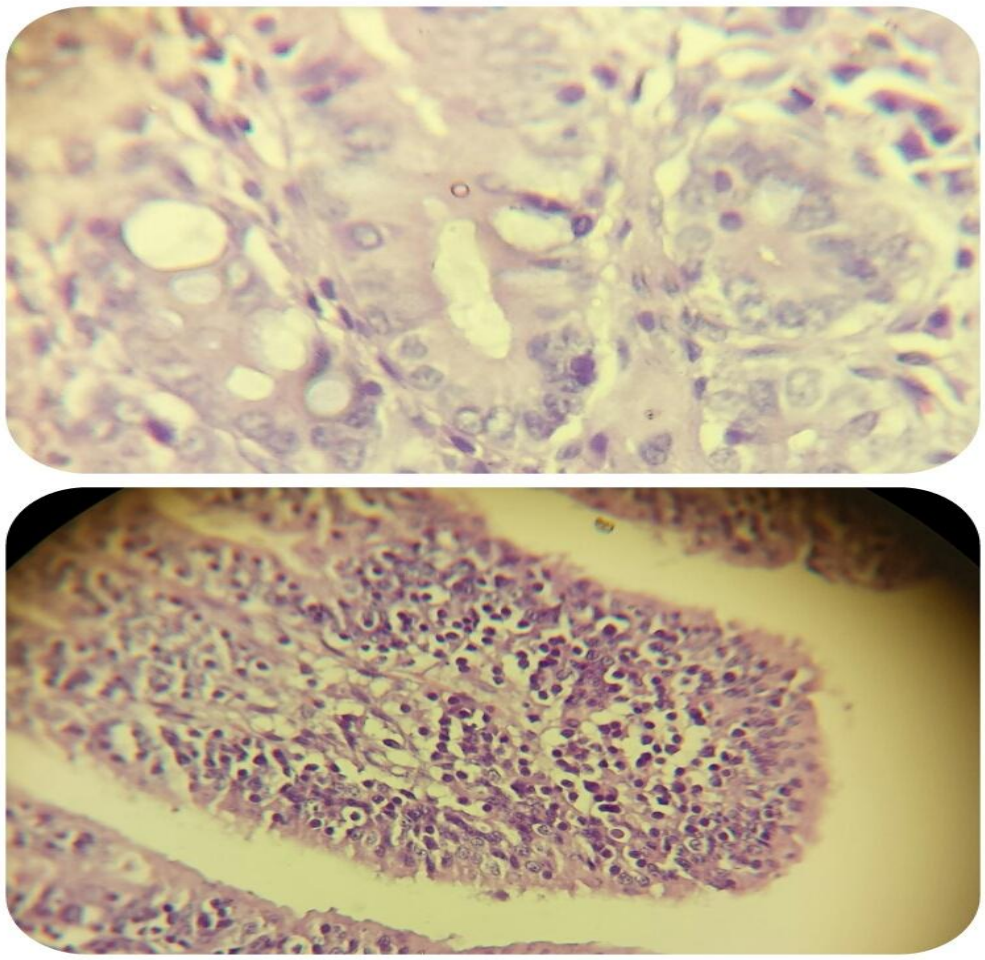

Figure (6-1) section in intestine of rabbits treated with curacumin

Histopathological analysis was carried out by hematoxylin/eosin method of the paraffin jelly rolled spleen. Samples presented with high-grade dysplasia (pre-cancerous lesion), highly infiltration with neutrophil 'rounding up' of cigar-shaped nuclei, also section exhibit highly proliferative cells with abnormal shape, where spleen tissues appear with blue color in comparative to pinky color for normal spleen (figure 3-3A,B), this changes due to same reasons for Pautz-syndrome (40), where exposure to carcinogenic material causing genetic defect in chromosome19, the gene known as STK11 (LKB1)(43) is a possible tumor suppressor gene (44).

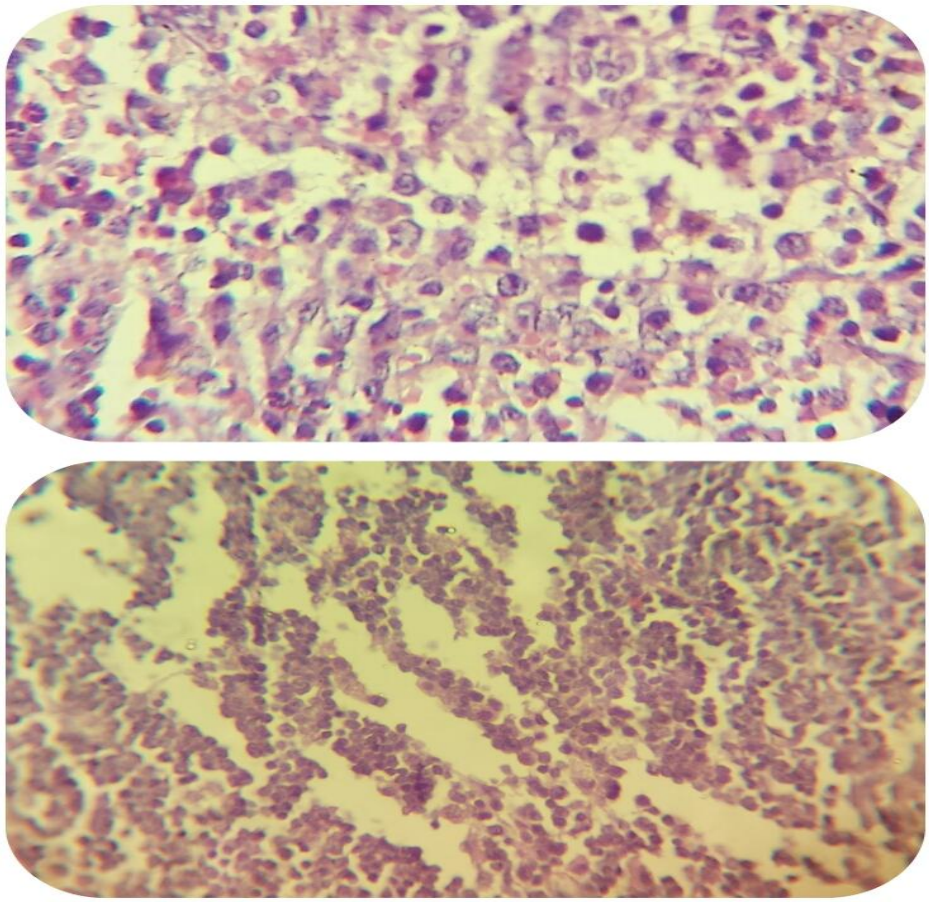

Figure (6-3) section of spleen in rabbits treated with curacumin

The distribution of curcumin in the various tissues namely, lung and heart is shown in figure (3-4). Upon oral administration of curcumin to rabbits group showed damage of aleviolar sac, air vacuoles formation, as well as infiltration of inflammatory cells in lung tissues. Recently, heart section revealed highly maceration in heart muscle (figure3-5). These results came in harmony with(45), they showed peak concentration of curacumin was observed at 6 h, after oral administration at dose (500 mg/kg).

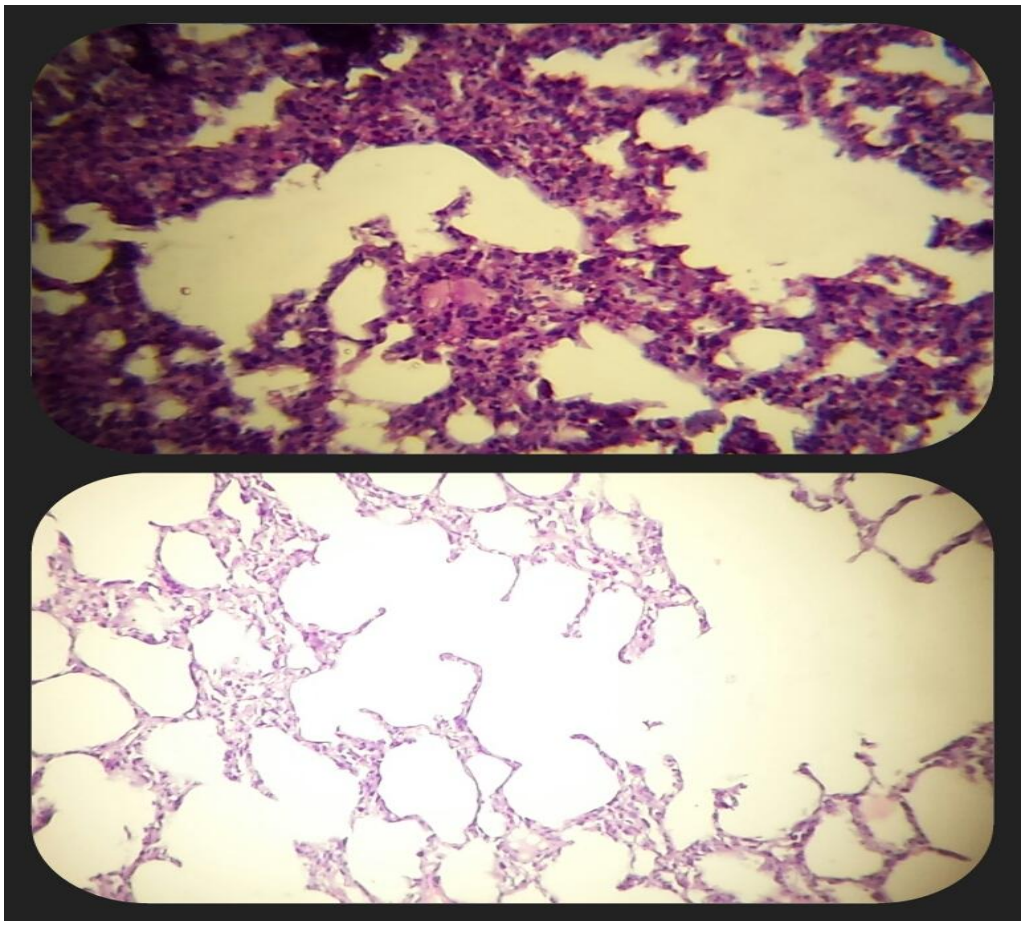

Figure (6-4) section of lung in rabbits treated with curacumin

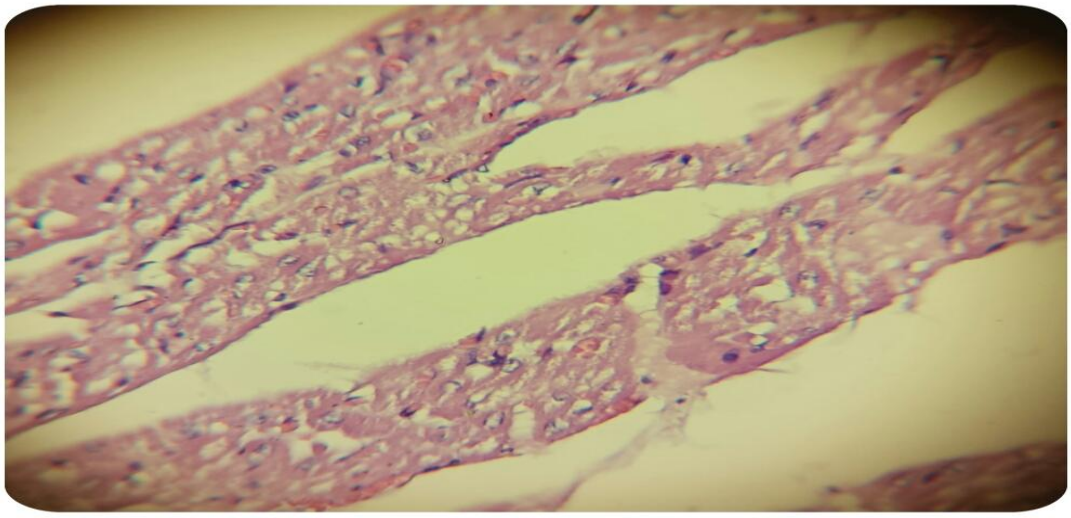

Figure (6-5)section of heart in rabbits treated with curacumin

Accusingly, previous study by (46)refers to curcumin stayed significantly longer in the body tissues when administered orally. Curcumin was also detected in the brain tissue at 24, 48 and 96 h with a maximum at 48 h.while(47) refered to highly subchronic toxicity of turmeric powder at 0.03,2.5 and 5.0 g/kg/day was investigated for 6 months in 96 wistar rats, where During subchronic treatment with turmericpowder, subcutaneous abscesses werefound in all treated groups, and the numbersof rats that developed abscess increased asthe dose increased. As

well as, there's interstitial fibrous in lungs, focal fatty change in liver, and focal calcification of renal medulla, acute tubular necrosis and focal chronic pyelonephritis in kidney. These results agreed with supported by (48) in which areduction in weight gain and food conversionefficiency was found in pig fed for 102-109days with 60-1551 mg/kg/day of turmeric.

7- Conclusions:

The results of this study confirm that curcumin (turmeric) had highly toxic effect in all internal tested organs specially (spleen and intestine). Where caused increase in proliferation cells as well as, increases their size and changes their morphological shape.

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