Synergistic Effect of Curcumin and Adriamycin on Liver Detoxification Enzymes in Hepatocellular Carcinoma-Induced Wister Strain Albino Rats.

Section A-Research paper

Synergistic Effect of Curcumin and Adriamycin on Liver Detoxification Enzymes in Hepatocellular Carcinoma-Induced Wister Strain Albino Rats.

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Abstract

Although hazardous industrial chemicals as well as pollutants in the air and water are causes of hepato cancer-causing potential in the former, additives to food and fungi are the primary causes of liver damage in the latter. Hepatocarcinoma is an important issue not only in wealthy nations but also in most developing countries. In Wistar strain rats, it has been discovered that daily dietary treatment of curcumin (2 mg/ml b.wt.) plus adriamycin (20 mg/kg b.wt.) significantly lowers the incidence of cancer caused by N-nitrosodiethylamine (DEN). Researchers have discovered that curcumin as well as adriamycin treatment is effective in reducing the liver microsomal the glutathione, cytochrome b5, cytochrome p450 NADPH-cytochrome C reductase, decreased glutathione-S-transferase, and glutamyl cysteine synthetase activities to a level that is statistically significant when measured in the hyperplastic a nodule or in the surrounding. Additionally, as compared to control rats, the study's curcumin and adriamycin treatments significantly reduced the microsomal activities of cytosolic glucose-6-phosphate dehydrogenase. Curcumin and adriamycin both have the potential to be anticancer medicines by modifying the activity of the enzymes that metabolise glutathione.

Key words: Hepatocellular Carcinoma, Curcumin, Adriamycin, Detoxification Enzymes

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Introduction

The terms tumor, neoplasia and cancer are used inter changeably. The word cancer implies a malignant neoplasm, usually but not always, of epithelial origin. Neoplasia represents, a defect in cellular differentiation, maturation and control of growth (1). The word "cancer" is used to refer to a broad range of malignant disorders, the treatment of which spans various medical specialties. Cancer spreads by invading tissues nearby and by metastasis to other locations. Regarding karyotype, morphology, immunogenicity, pace of development, capacity for metastasis, and susceptibility to antineoplastic medicines, tumours can exhibit significant heterogeneity (2). In particular in Africa and East Asia, hepatocellular carcinoma (HCC) is a frequent neoplasm. Aflatoxin use and hepatitis B infection are to blame for the high prevalence of HCC in China and Africa, respectively (3). Hepatocarcinoma is an important issue not only in wealthy nations but also in most developing countries. The liver is particularly vulnerable to carcinogenic damage since it is the primary place in the body where ingested material is metabolised. Furthermore, hepatocarcinoma is seldom discovered at an early stage and, once discovered, therapy often has a poor prognosis because to the high tolerance of the liver (4). The malignant subtypes of these neoplasms include perisinuoidal (Ito) cell sarcomas, angiosarcomas, hepatocellular carcinoma, and cholangiocellular carcinoma. In empirical chemical hepatocarcinogenesis models, the progressive cellular and molecular alterations prior to these neoplasms have been thoroughly described. In experimental hepatocarcinogenesis, altered hepatocyte foci that are preneoplastic occur weeks or months before hepatocellular adenoma and HCCs do (5).

The complicated procedure of carcinogenesis is brought on by deviations from the typical cellular growth patterns. The presence of multiple changed or "resistant" cells during start in the resistance hepatocyte experiment on liver carcinogenesis in rats is a crucial event that, following promotion, causes the development of hepatocyte clusters with a distinctive altered phenotype. Most of these hepatocyte lesions go away or re-differentiate into liver that

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seems normal, but a small number of "persistent nodules" turn into cancerous tumours (6). The equilibrium among cell death and multiplication is upset in the hepatocyte granules leading to an overall rise in cell proliferation. This process alters in persistent nodules, where increased cell death balances off increased cell proliferation, causing persistent nodules to develop more slowly. This equilibrium is once again upset when cancer develops due to the neoplastic tissue tumours' faster development rate.

Table 1 : Classification of primary liver tumors

S.No.	Type	Benign	Malignant
1.	Epithelial tumors	Liver cell adenoma, bile duct adenoma and bile duct cystadenoma	HCC (liver cell) cholangio carcinoma bile duct cystadenocarcinoma and hepato blastomas.
2.	Non-epithelial tumors	Hemangioma	Angio sarcoma Embryonal, rhabdomyo, leiomyo and fibrosarcomas.
3.	Tumor-like lesions	Cysts mesenchymal, hemertona, focal nodular hyperplasia, peliosis, teratoma	-

Many different organic natural substances that have been shown to have mutation (or) cancer-causing properties have been shown to be present in human food products, medications, and extracts from plants (8). Among these are many antibiotics, hazardous the mycotoxins (aflatoxin, PR-toxin, T-Z toxin), the substance chemicals, safrols, alkaloid compounds, the tannins, cycasin, urethane, psoralens, alkyl isothiocyanates, hazards of spruce (Pteridium equilinum), aristolochic acid (AA), and isatidin. As the fundamentals of chemical carcinogenesis have been clarified, the threat of cancer brought on by these substances has gained more attention (9). A common nitrosamine that may be found in a variety of processed foods and cigarettes is N-nitrosodiethylamine (DEN). These substances can also be created in living things under physiological circumstances (10, 11). The usage of

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tobacco-related goods, cosmetics, pharmaceuticals, and agricultural pesticides can expose people to preformed N-nitrosoamines through their food, specific work settings, and occupational exposure (12, 13). One of the most significant cancer-causing substances in this family, N-nitrosodiethylamine, largely causes liver tumours. It is commonly acknowledged that nitrosamines cytotoxic, mutagenic, and carcinogenic action is dependent on the metabolic stimulation of nitrosamines by cytochrome P450 enzymes that are exposed to reactive electrophiles (14).

The rhizomes of the turmeric plant, Curcuma longa Linn, contain the polyphenol curcumin (diferuloyl methane). It is a spice with a yellow hue that is frequently used in Indian cuisine. In Asian medicine, turmeric has long been used as an anti-inflammatory treatment in the form of a herbal powder. Turmeric is used in traditional eastern medicine to treat rheumatism, sinusitis, an eating disorder, sneeze diabetic wounds, hepatic diseases, and biliary problems (15). A number of ROS, such as the superoxide anion, hydroxy radical, a singlet oxygen, nitric oxide, and peroxynitrite are effectively neutralised by curcumin. Haemoglobin, DNA, and lipids can all be protected by curcumin against oxidative damage. Demethoxy curcumin and bisdemethoxy curcumin are not as effective in scavenging superoxide anion as pure curcumin (16). The ROS-producing enzymes cyclooxygenase and lipoxygenase are both effectively inhibited by curcumin in mouse epidermis (17). Skin, colorectal, dental, forestomach, and breast cancers are only a few of the areas in which curcumin slows carcinogenesis. Curcumin's anti-initiation and antipromotional properties have been linked to its ability to prevent tumour growth. While the antipromotion impact may be achieved by inhibiting cell proliferation or apoptosis-promotion of the newly started cells, the anti-initiation activity may derive from its capacity to limit the production of DNA damage. Curcumin has been shown to be protective against N-diethyl the nitrosamine (DEN)-induced HCC development in mice by Chuang et al. (18). In mouse epidermis, curcumin prevented the development of tumours caused by benzo(a)pyrene (Bap) and 7,12-dimethyl

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benz(a)anthracene (DMBA). When applied topically, curcumin significantly reduced the development of skin tumours in DMBA-induced mice. The amount of duodenal tumours caused by N-ethyl-N-nitro-N-nitrosoguanidine (ENNG) in each animal was reduced when curcumin was added to the diet. In mice and rats, the consumption of curcumin in the diet reduced the amount of colon tumours brought on by azoxymethane (AOM) (19). Curcumin has been shown in recent research to have strong anti-metastatic impact in mice. Cellular invasion and migration were measured using an in vitro experiment, either with or without the Matrigel matrix. To measure the production of matrix metalloproteinase-9 (MMP-9), gelatin-based zymography was used (20). According to Lin, curcumin at a concentration of 10M prevented SK-Hep-1 cells from migrating and invading by 17.4% and 70.6%, respectively. Huh 7, a less aggressive human carcinoma of the liver cell line, and SK-Hep-1 had significantly different levels of MMP-9 secretion. Additionally, curcumin suppressed MMP-9 production in SK-Hep-1 in a dose-dependent way in tandem with its anti-invasion effects. According to Lin's findings, curcumin significantly inhibits MMP-9 release in SK-Hep-1 cells, which is a key component of its anti-invasion function (21). Plant compounds have long been recognised to have anticancer properties. Many of the antineoplastic substances in use today were first derived from plants.

The medicine doxorubicin, also known as adriamycin, is regarded as one among the most successful ones for treating sarcomas. A variety of human neoplasms are treated with adriamycin (ADR), an anthracycline anti-neoplastic drug (22). Acute leukaemia, lymphoma, and a variety of solid human tumours have shown it to be effective (23). According to Koukovrakis et al. (24), adriamycin is one of the most successful medications for treating sarcomas. Stealth liposomal medications have a long circulation half-life because their small size and structure prevent extravasation. The medication is therefore specifically deposited in tissues with higher permeability to blood vessels, which includes tumoral tissue. The liposomal formulation is appealing when combined with radiation because of its selective

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accumulation (24). Anthracyclin antitumor antibiotic ADR's mode of action has been well investigated. The Chromatin-ADR interactions have been shown to impair DNA replication, transcription, and repair, potentially by interfering with DNA's template function. This medication was discovered to have a strong attraction for DNA. Combination chemotherapy was used to test the idea that disrupting a tumour cell's metabolism simultaneously at several sites would have a more significant impact on it than disrupting just one metabolic zone. This method of treating lymphomas resulted in dramatic outcomes (25). In 15-20% of patients, the four-drug regimen of mustard nitrogen, vincristine, procarbazine, and prednisalone produced full remissions. The odds of remission for acute leukaemia rose from 20% to 90% with similar combination treatment. Combination chemotherapy has been proven to be beneficial in many different malignant illnesses during the past 20 years, including carcinomas of the liver, breast, ovary, lung, and various children tumours. It is ideal for each medication in the treatment combination to have a unique mode of action, be efficient when used alone, and have qualitatively distinct toxicities so that each may be administered at or close to their unique maximum tolerated dosages (25). A significant barrier to the therapeutic use of adriamycin in the treatment of cancer is cardiac oxidative damage. A number of processes, including peroxidation of membrane lipids, free radical production, damage to mitochondria, and iron-dependent reactive damage to biological macromolecules, are involved in ADR-induced cardiotoxicity (22). Recent research has shown that ADR biologically produces oxygen free radicals that interact with DNA in vivo and in vitro, limiting the production of both DNA and RNA. Following ADR treatment, higher levels of lipid peroxidation and accelerated free radical production in the circulatory system have been seen, and these processes have subsequently been linked to cardiac damage. ADR-induced cardiotoxicity is also characterised by suppression of oxidative phosphorylation, reduced ATP production, and other symptoms (23).

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Despite recent improvements in cancer detection and treatment, the illness still ranks among the leading causes of death globally. Chemotherapy is necessary in order to treat cancer effectively because our usual weapons, such as surgical and radiation, cannot be said to have total control over the disease. The hunt for anti-cancer medications with minimum cytotoxicity towards normal cells is currently ongoing as the majority of anti-cancer therapies generate adverse reactions at their therapeutic level.

2. MATERIALS AND METHODS

2.1 Animals

The Frederick Institute of Plant Safety and Toxicity (FIPPAT), Padappai - 601 301, Chennai, India provided male Wistar strain albino rats (50–60 days old). They were given access to free, clean drinking water as well as regular rat food sold by Hindustan Lever Limited in Mumbai, India. The following substances were purchased from Sigma Chemical Company, St. Louis, MO, USA: N-nitrosodiethylamine (DEN), phenobarbital (PB), bovin serum albumin, bathophenanthroline, 6-phosphate of glucose, -glutamyl para-nitroaniline, reduced glutathione, and adenosine triphosphate. 1,2,4-amino naphthol sulphonic acid and nicotinamide dinucleotide (NAD+, NADH) were purchased from Fluka, Switzerland. We bought sodium pyruvate, 2,4-dinitro phenyl hydrazine, and 5,5-dithiobis from BDH Division in England. Sodium selenite, thiobarbituric acid, and chromotropic acid were produced from Loba Chemie, Mumbai, India. Pharmacia & Upjohn India Pvt. Ltd., in Haryana, India, was where the adriamycin was purchased. Herbo Organics Private Limited, SIDCO Industrial Estate, Kakkallur 602 003, India, was the source of the curcumin. The highest possible quality and analytical grade were employed for all other compounds and reagents. All other chemicals, including solvents, were purchased from Glaxo Laboratories, SD Fine Chemicals, and Sisco Research Laboratories Pvt. Ltd., all of which are located in Mumbai, India. They are all of high purity and analytical quality.

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2.2 EXPERIMENTAL DESIGN

Six groups of six animals each were formed from the animals, as follows: Group I: Animals in control. Group II: Animals prone to hepatocellular carcinoma (den; 200 mg/kg body weight in saline; one intraperitoneal injection at 10 weeks of age). Phenobarbital (0.05%) was provided as a carcinogenesis promoter two weeks after DEN injection. Up to 14 weeks in a row, the promoter was added to the rat food. Animals with hepatocellular cancer in Group III were given Adriamycin (20 mg/kg b.wt., i.p.) for two weeks in a row. Group IV: Animals with hepatocellular cancer who were given curcumin (200 mg/kg b.wt. orally) for two weeks in a row. Animals with hepatocellular cancer in Group V were given the same curcumin and adriamycin treatment as before. Group VI : Curcumin alone treated animals (as above).   The rats were killed by cervical decapitation at the conclusion of the treatment period, and the serum was extracted from the blood that had been collected. Physiological saline was used to promptly remove the liver and kidney and wash them. They were blended into a 10% homogenate using 0.1 M Tris-HCl buffer at pH 7.4. For the estimate, liver and kidney homogenates were employed. For histological analysis, a piece of the kidney and liver tissues was embedded in 10% formalin.

The weight of the tumour was calculated using the Geren et al. (26) approach. A prolate ellipsoid with one long axis and two short axis was thought to represent the shape of the solid tumour that resulted. Vernier callipers were used to measure the two short axes. The tumour weight was determined by multiplying the tumor's length by its square measure of breadth, then dividing the result by two.

$$\text{Tumor weight (g)} = \frac{length \ x \ width}{2}$$

The weight of the tumour as determined by Vernier calliper measurements and its real weight were found to be quite similar.

2.4 ALPA-FETOPROTEIN (AFP) ESTIMATION

After letting the collected blood coagulate, the serum was extracted (27). The samples and reagents were combined after being brought to room temperature. Serum sample, control,

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and standards totalling 25 l were then injected into the designated wells. The allotted wells were then immediately filled with 100 l of 0 IU/ml AFP standard solution, which was subsequently incubated for 30 minutes at the ambient temperature. The wells were washed with running water five times after the mixture for incubation was removed. Then each well received 100 l of an enzyme conjugate, which was incubated once again for thirty minutes at room temperature. Each well received 100 l of the first solution and 100 l of solution B before being incubated at room temperature for 10 minutes. The addition of one teaspoon of one molecule of H_2SO_4 to each well halted the process, and the optical density at a wavelength of 450 nm was measured using a microwell reader. In IU/ml, the AFP concentration is indicated.

2.5 GLUTATHIONE METABOLISING ENZYME ESTIMATION

2.5.1 Reduction in Glutathione

The technique of Moron et al. (28) was used to calculate the total reduced glutathione. With 5% TCA, 0.1 ml of the homogenate of the tissue was precipitated. Centrifugation was used to separate the precipitated. A final amount of 3.0 ml of 2.0 ml of DTNB in a phosphate buffer solution containing 0.2 M was added to a portion of the supernatant. At 412 nm, the absorbance was measured against a blank that contained TCA rather than the sample. To ascertain the glutathione content, a variety of standards were also processed in a similar manner. The glutathione concentration was given as n moles/g moist tissue.

2.5.2 Glutathione-S-transferase

The technique developed by Habig et al. (29) was used to measure glutathione S-transferase. 0.1 ml of CDNB, 1.7 millilitre of water, and 0.1 ml of an enzyme source were added to 1.0 ml of phosphate buffer. 0.1 ml of GSH was incorporated after five minutes of incubating at 37C, and the optical density was immediately monitored for 3 minutes. As a control, a full test combination devoid of enzyme was employed. A Shimadzu spectrophotometer was used to measure optical density at 340 nanometers. The amount of moles of CDNB of conjugate produced per minute/mg protein was used to express the glutathione S-transferase activity.

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2.5.3 Glutathione Reductase

The Staal et al. (30) technique was used to measure glutathione reductase. With distilled water, the reaction mixture comprising 1 ml of phosphate buffer, 0.5 millilitres of EDTA, 0.5 ml of GSSG, and 0.2 ml of NADPH was raised to 3 ml. After 0.1 ml of homogenised tissue was added, a shift in the optical density at 340 nm was observed for 2 minutes at intervals of 30 seconds. Under incubation conditions, the enzyme activity was measured in moles of NADPH oxidised per minute per mg of protein.

2.5.4 Glutamyl Cysteine Synthetase

The technique developed by Mooz and Meister (1971) was used to measure the activity of - glutamyl cysteine synthetase. A portion of the homogenised was incubated for an hour at 37C in the reaction combination (final quantity, 1 ml) that also contained 0.15 ml of ATP, 0.15 ml of $MgCl_2$, 0.15 ml in sodium glutamate, and 0.10 ml of cysteine. By adding 1 ml with 10% TCA, the process was stopped. The amount of freed inorganic phosphorus was measured in the supernatant following centrifugation using the Fiske and Subbarow technique (31).

2.5.5 Glucose-6-phosphate Dehydrogenase

The Beutler technique (32) was used to measure this enzyme. 0.2 ml magnesium chloride, 0.2 ml NADP, 0.36 ml water, and 0.2 ml of enzymes were added to 0.2 ml of Tris-HCl buffer. 0.2 cc of glucose-6-phosphate solution was added to start the reaction after 10 minutes had passed. At 340 nm in a Kontron spectrometer set to a temperature of 25C, the rise in the density of light was observed. The enzyme's activity was measured in units/mg protein, where one unit is equal to the quantity of the enzyme needed to produce a variation in optical concentration of 0.01/min.
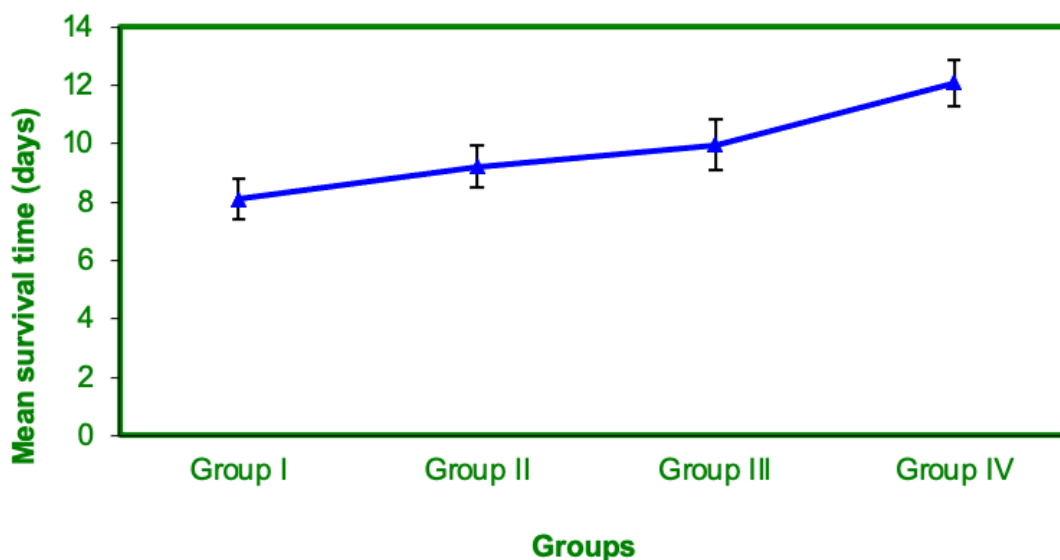
Results

The tumour weight in HCC-bearing control and treated mice is shown in Figure 1. When compared to untreated HCC rats, the tumour weight was shown to be considerably (p 0.05) decreased with adriamycin therapy. In HCC-bearing mice treated with curcumin, the

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tumour weight was similarly considerably (p0.01) decreased. The combination use of adriamycin and curcumin was shown to be more effective at causing tumour regression (p 0.001).

Figure 1: shows the effect of curcumin on body weight in control & experimental animals
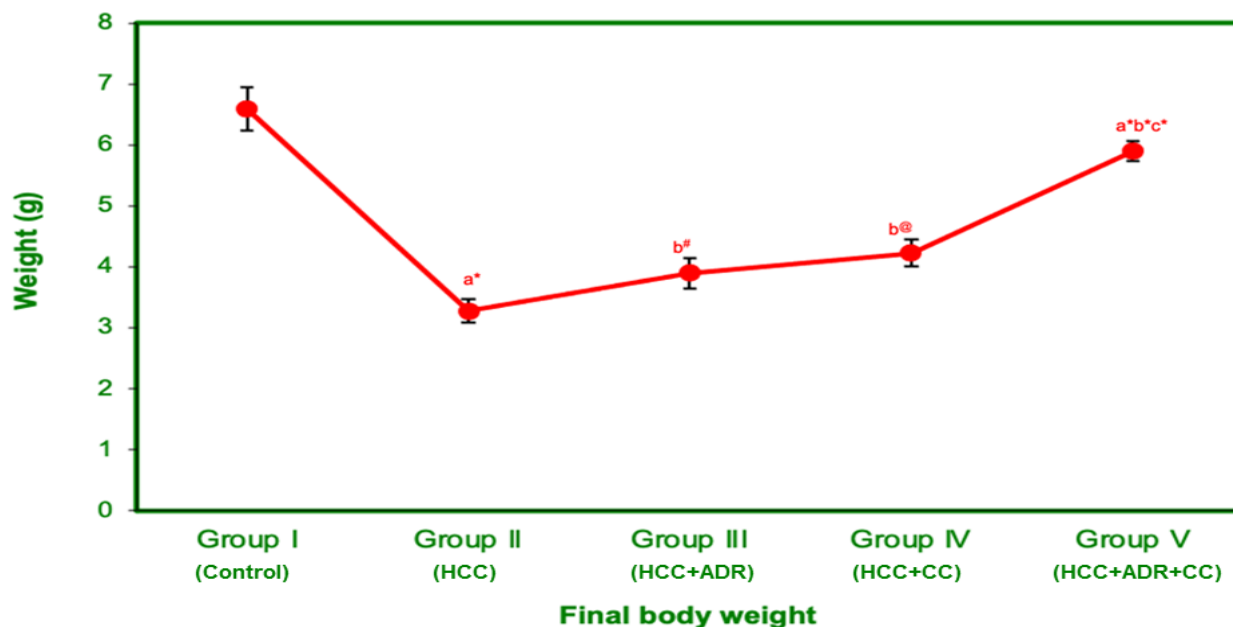

The average survival time of hepatocytes bearing control and treatment animals is shown in Figure 2. Among all the treated animals, it was discovered that the mean survival time had risen. When compared to either adrimycin or curcumin treated rats, combined therapy with adriamycin and curcumin dramatically (p0.001) increased the mean survival time.

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The blood glucose levels in experimental and control mice are shown in Figure 3 respectively. As compared to control (Group I) animals, it was discovered that the blood glucose level was considerably (p 0.001) lower in HCC-bearing Group II mice who had not received any treatment than in Group III animals that had received adriamycin treatment. When compared to HCC-bearing (Group II) mice that were not treated, the blood glucose level in the curcumin-treated (Group IV) and adriamycin and curcumin-treated (Group IV) animals returned to almost normal (p0.001).

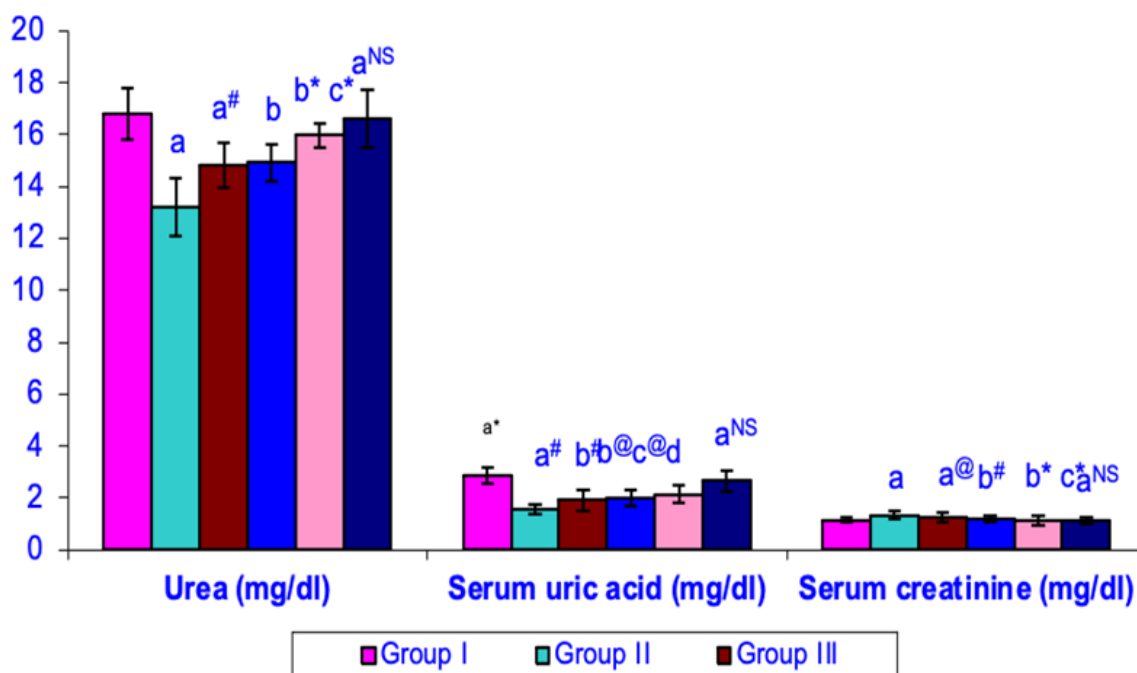**Figure 3: The level of blood glucose in control and experimental animals**
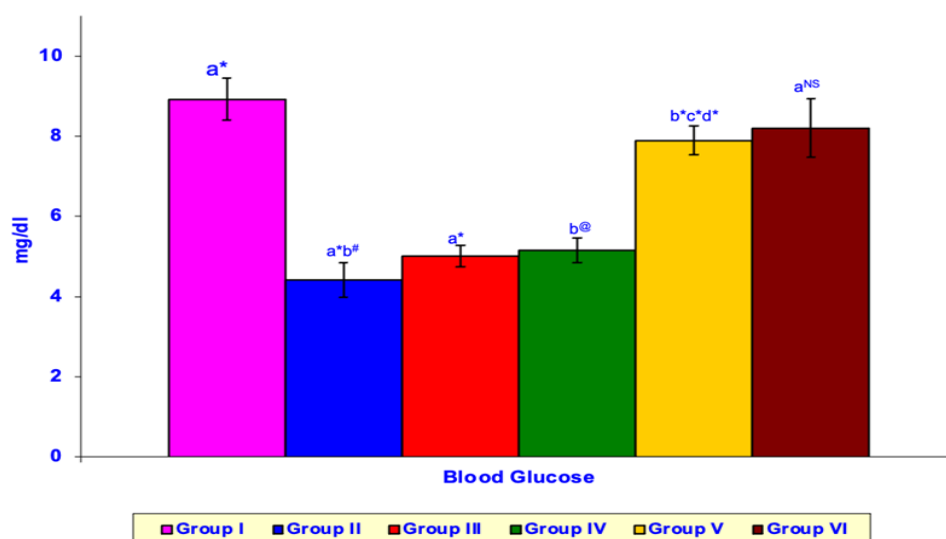
Values are expressed as mean $\pm$ SD in six animals of each group
a - Group II, III and VI compared with group I; b - Group III, IV and V compared with Group II; c - Group V, compared with group III; d - Group V, compared with Group IV

*p<0.001, @p<0.01, #p<0.05 and NS - Not significant

The total amounts of urea, uric acid, and creatinine in the blood and serum of both the experimental and control groups are shown in Figure 4. Untreated HCC carrying mice and group III adriamycin treated HCC bearing animals had elevated (p0.001) levels of creatinine, although urea and uric acid levels were lowered (p0.001) in comparison to group I control animals. When compared to group III's adriamycin alone treated HCC bearing mice, group V's adriamycin and curcumin treated animals saw their levels of urea, uric acid, and creatinine return to close to normal (p0.001).

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Figure 4 shows the levels of blood urea, serum uric acid, and serum creatinine in control & experimental animals
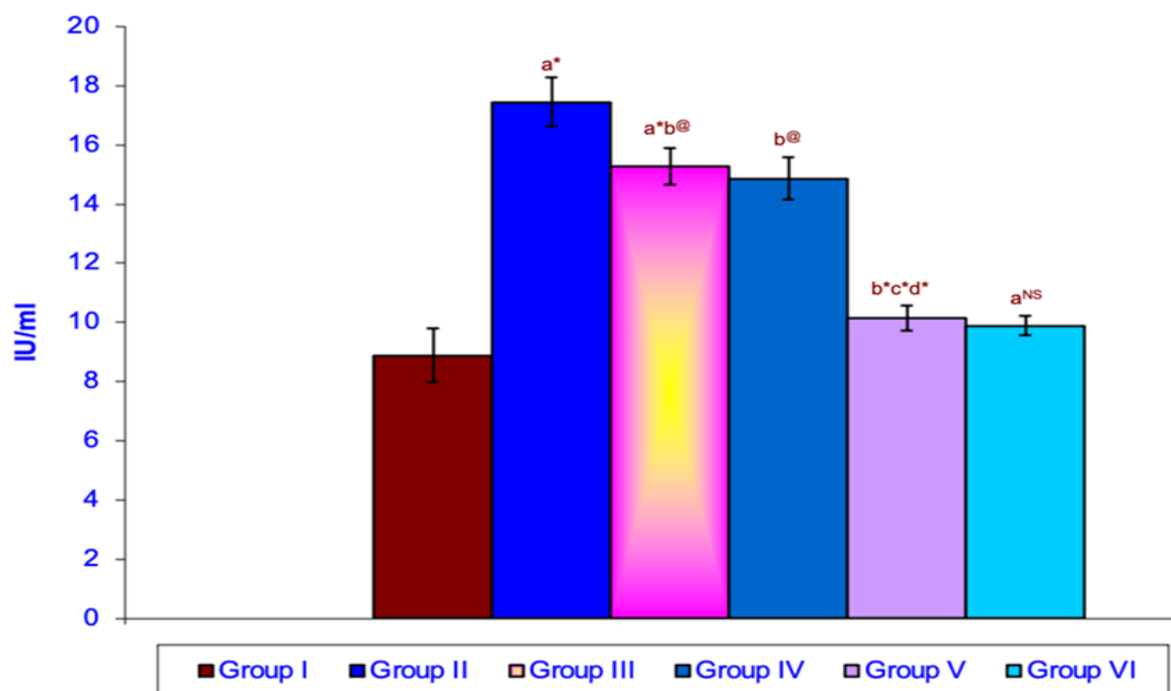

Values are expressed as mean $\pm$ SD in six animals of each group
a - Group II, III and VI compared with group I; b - Group III, IV and V compared with Group II; c - Group V, compared with group III; d - Group V, compared with Group IV
*p<0.001, @p<0.01, #p<0.05 and NS - Not significant

The serum alpha-fetoprotein levels of experimental and control mice are shown in Figure 5 respectively. In hepatocellular carcinoma-bearing animals compared to healthy control animals, the AFP level rose by 11 times. This level was 10X lower in group V animals treated with adriamycin and curcumin.

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Figure 5 The levels of AFP in serum of control and experimental animals

Values are expressed as mean $\pm$ SD in six animals of each group
a - Group II, III and VI compared with group I; b - Group III,IV and V compared with Group II; c - Group V, compared with group III; d - Group V, compared with Group IV

*p<0.001, @p<0.01, #p<0.05 and NS - Not significant

The glutathione metabolising enzyme activity in the livers of control and experimental animals are shown in Table 2 and Figure 6. As compared to group I control animals, GST, GSH, GR, -GCS, and G6PD activities in group III animals having HCC were shown to be reduced (p0.001). When compared to group III animals treated with adriamycin alone, administration of adriamycin with curcumin in group V animals dramatically (p 0.001) reversed the enzymes activity. As compared to group I animals, group VI animals showed no discernible differences.

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Table 2: The activities of glutathione metabolising enzymes such as GST, GR and Glutathione in liver of control and experimental animals

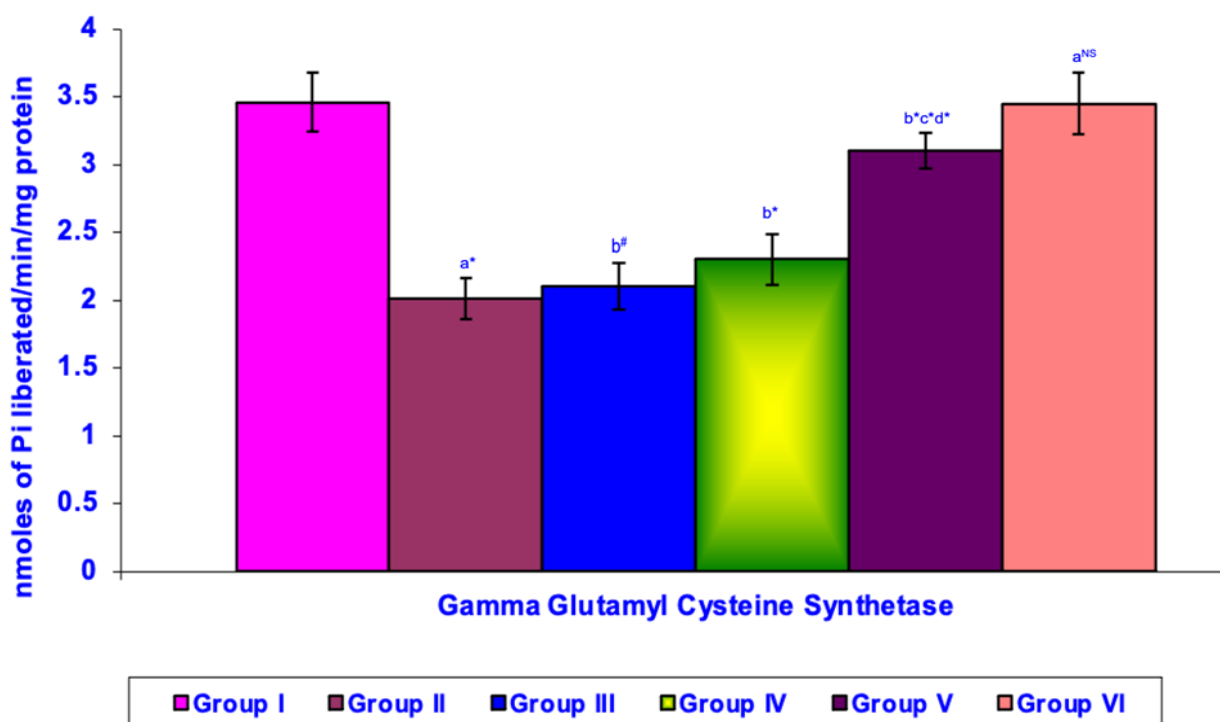| Parameters	Group I (Control)	Group II (DEN induced)	Group III (DEN + ADR)	Group IV (DEN + CC)	Group V (DEN + ADR+CC)	Group VI (CC alone)
Glutathione-S-transferase (µg of thio ester formed/min/mg protein)	0.22 ± 0.03	$0.13 \pm 0.01^{a*}$	$0.17 \pm 0.07^{a*bNS}$	$0.18 \pm 0.05^{b*}$	$0.20 \pm 0.04^{b*c*d@}$	0.21 ± 0.09^{aNS}
Glutathione reductase (µ moles of NADPH oxidised/min/mg protein)	5.98 ± 0.38	$3.12 \pm 0.32^{a*}$	$3.40 \pm 0.27^{a*bNS}$	$4.13 \pm 0.25^{b*}$	$5.67 \pm 0.19^{b*c*d@}$	5.84 ± 0.21^{aNS}
Glutathione (µg of GSH/mg protein)	4.07 ± 0.40	$2.72 \pm 0.23^{a*}$	$3.18 \pm 0.29^{a@b\#}$	$3.67 \pm 0.45^{b@}$	$3.85 \pm 0.27^{b*c*d@}$	3.92 ± 0.63^{aNS}

a - Group II, III and VI compared with group I ; b - Group III, IV and V compared with group II
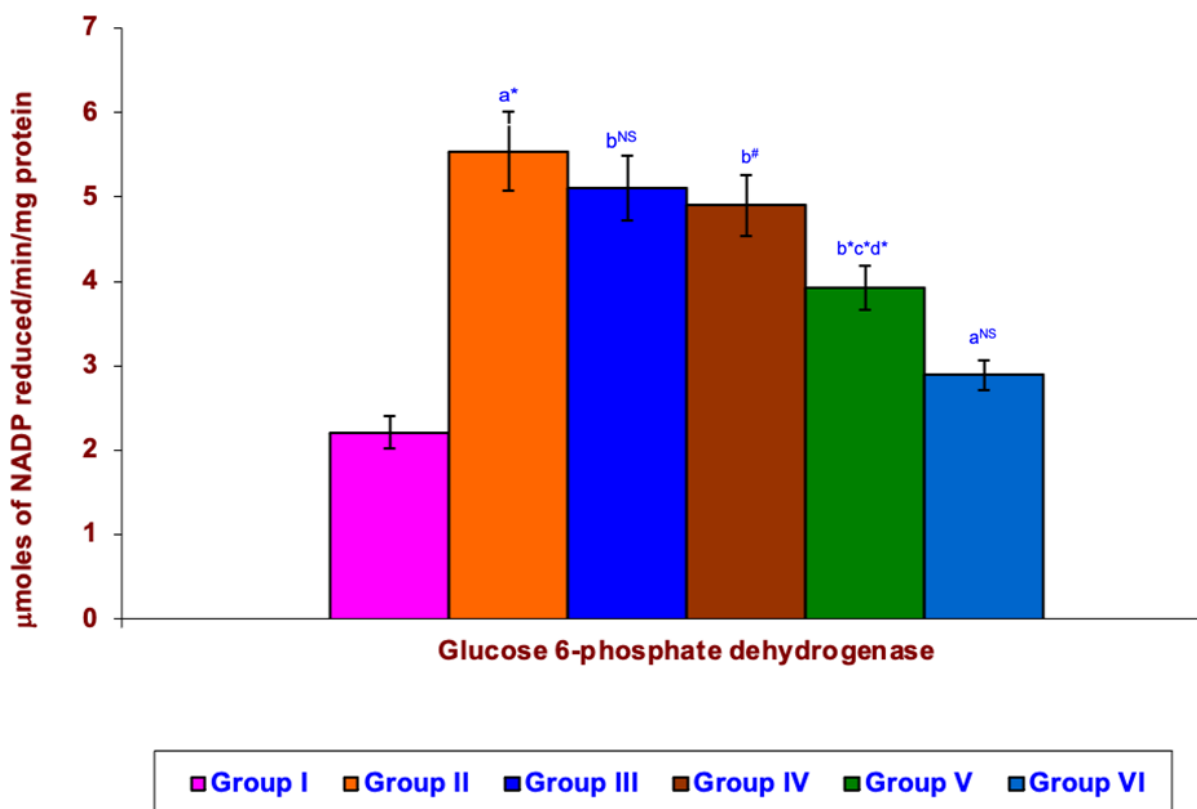c - Group V, compared with group III; d - Group V, compared with group IV
Values are expressed as mean $\pm$ SD in six animals of each group.

$^{*}p < 0.001$, $^{@}p < 0.01$, $^{\#}p < 0.05$ and NS - Not significant.

Figure 6 The activities of γGCs and G-6PDH in liver of control and experimental animals

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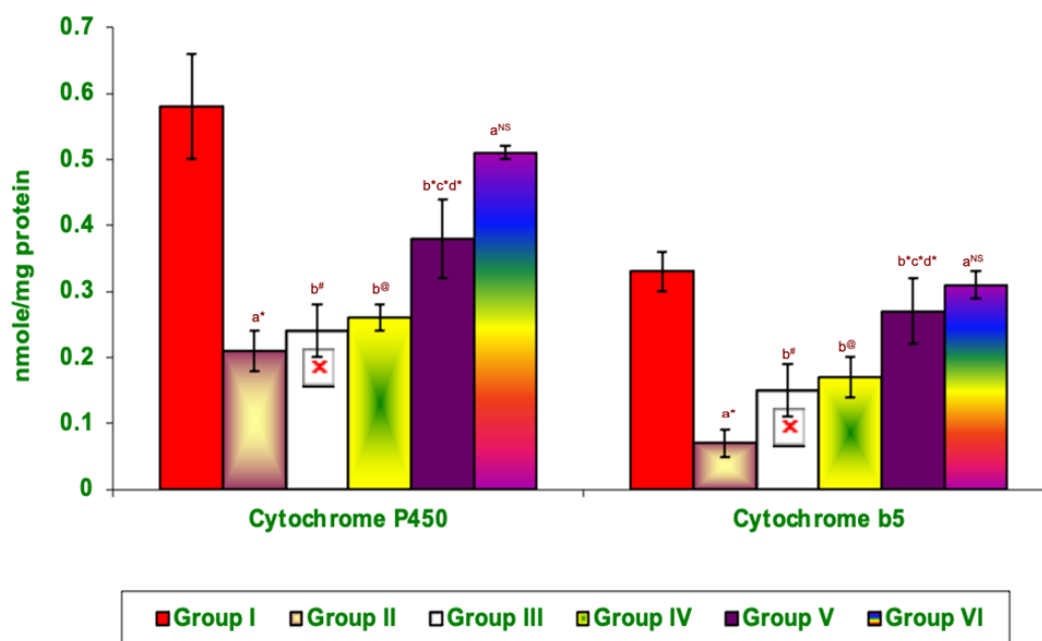
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Values are expressed as mean $\pm$ SD in six animals of each group
a - Group II, III and VI compared with group I; b - Group III,IV and V compared
with Group II; c - Group V, compared with group III; d - Group V, compared with Group IV

$^{*}p<0.001$, $^{@}p<0.01$, $^{\#}p<0.05$ and NS - Not significant

The quantities of cytochrome P450 and cytochrome B5 in the livers of both the control and experimental groups are shown in Figure 7. As compared to group I animals, the reduced glutathione concentrations were lower (p 0.001) in HCC-bearing group II animals. When group V (adriamycin + curcumin) animals were compared to group II and group III throughout the investigation, the glutathione content rose (p 0.001), however group VI (curcumin alone) animals were not statistically different when compared to group I animals.

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Figure 7 The levels of cyt p450 and cyt b5 in liver of control and experimental animals

Values are expressed as mean ± SD in six animals of each group
a - Group II, III and VI compared with group I; b - Group III,IV and V compared with Group II; c - Group V, compared with group III; d - Group V, compared with Group IV

*p<0.001, @p<0.01, #p<0.05 and NS - Not significant

Discussion:

Hepatocarcinoma is an important issue not only in wealthy nations but also in most developing countries. The liver is particularly vulnerable to carcinogenic damage since it is the primary place in the body where ingested material is metabolised. Additionally, hepatocarcinoma is seldom discovered at an early stage and, once discovered, therapy often has a bad prognosis because of the high tolerance of the liver. It is most likely caused by the massive tumour mass's need for glucose and a lack of adequate liver tissue to continue glucose production. Even with a high-carbohydrate diet, corticosteroids, and diazoxide, it is frequently challenging to maintain blood glucose levels. Both the liver tissue around the tumour and the last enzyme in the gluconeogenic pathway, glucose-6-phosphatase, are decreased (33). An area of intense scientific interest is the utilisation of phytochemicals with

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cytotoxic characteristics as an adjuvant therapy for cancer (34). When administered intraperitoneally, the antitumor effects of curcumin and adiramycin may be seen. Curcumin administration resulted in much more tumour shrinkage than ADR treatment, indicating that curcumin has an anticancer impact on hepatocellular carcinoma-bearing mice. The tumour regression with curcumin therapy was visible, by itself. The prevalence and size of hyperplastic nodules are correlated with hepatocarcinoma in both experimental and human illness, according to a substantial body of research. Long term infections, alcoholic and non-alcoholic prolonged liver damage, and oxidative stress are all known to contribute to the development of HCC. Phase 2 enzymes, such as conjugating and antioxidant enzymes like the enzyme glutathione S-transferase (GST), UDP-glucuronosyltransferase (UGT), and NAD(P)H: quinone oxidoreductase (NQO1), have cytoprotective effects by removing mutagens and carcinogens and boosting cellular resistance to oxidative stress [36]. A transcription factor called nuclear component-E2-related factor 2 (Nrf2) activates an antioxidant response component (ARE) in the regulatory parts of several genes that code for these cytoprotective enzymes (35,36). In light of this, curcumin and ADR, which were found to decrease nodule formation and promote their retreat in our study, may be crucial for the prevention of cancer, especially given that chronic nodules are easily recognisable and have a low chance to regress spontaneously. Animals in a group that had received curcumin treatment had longer survival times. These findings show that curcumin has anticancer properties against hepatocellular carcinoma. The metabolic processes for disposing of ammonia, the hazardous by product of nitrogen metabolism, are closely tied to urea synthesis. Reduced hepatic urea production may be the cause of the decreased urea level in patients with liver cancer. This lower blood urea nitrogen level is a sign of functional hepatocellular malfunction. According to Mc-Intyre and Rosalki (36), increased metabolic abnormalities in cancer conditions are caused by decreased urea production and a corresponding reduction in ammonia elimination. The metabolic by product of purine

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metabolism, uric acid, has demonstrated to be an effective antioxidant, particularly reactive with radicals that are free and hypochlorous acid (37). Studies revealed that hepatocytes have the capacity to transdifferentiate into true, fully functional cholangiocytes under severe circumstances, a process controlled by TGF- signalling. This might be a viable mechanism for the production of iCCA from hepatocytes in the setting of chronic liver injury, even if it is presently unknown whether such an event could take place during liver carcinogenesis (38).

Ascorbate is shielded from oxidation by cupric ions as well as from oxidation brought on by iron by ureate. Urate has both chain-breaking and preventative antioxidant properties, according to Wayner et al. (39,40). The decreased amount of uric acid in HCC circumstances may be attributable to enhanced uric acid utilisation against increased free radical generation, a hallmark of cancer situations. The lowered levels of urea in the blood and serum uric acid were practically restored back to near normal levels when the animals received a combined therapy of adriamycin and curcumin, demonstrating the protective effects of curcumin on adriamycin-induced nephrotoxicity and hepatotoxicity. Renal function is measured by serum creatinine. It is created endogenously by the breakdown of tissue creatine, and the tissue damage may be the cause of the rise in blood creatinine. The rate of glomerular filtration determines how much creatinine is eliminated, and when it doesn't match the rate of synthesis, serum creatinine levels increase (41). In the current investigation, we also noted that rats with HCC had higher creatinine levels. The raised levels of creatinine in the blood were practically reduced back to normal levels when the mice received a combined medication of curcumin. Hypoglycemia is a significant consequence of both cancer treatment with adriamycin and is also a key metabolic fuel for quickly expanding tumours. In comparison to healthy cells, cancer cells catabolize glucose more quickly, and in laboratory animals, malignant tumours operate as a glucose trap (41). Reduced blood glucose levels in tumour conditions showed that there is not enough glucose available to support both the host and the tumour. In addition to being transferred away from tissue hosts for direct tumour use,

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glucose serves as a crucial precursor for the production of other compounds that could be necessary for tumour development. Animals with tumours show this elevated metabolic requirement for glucose (42). The blood glucose level may have been returned to almost normal in the adriamycin and curcumin treated rats as a result of the anticancer activity of curcumin. The excessive use of glucose is normalised in this category of animals as a result of the lowered tumour load. In hepatoma-bearing animals (42), glutathione-S-transferase (GST) and glutathione reductase (GR) activity were elevated. It is well known that the placental form of GST serves as an effective marker for the various phases of hepato carcinoma (43). A class of intracellular proteins known as glutathione-S-transferases is engaged in cellular defence versus xenobiotics and carcinogens that have an electrophilic centre by conjugating them with reduced glutathione to produce molecules that are more soluble and easier to extract (43). Because they promote the production of drug GSH conjugates, increasing levels of GSH and GST activity have been considered by a number of authors to be relevant to human cancer as indications of treatment resistance (44). GSH is considered to be engaged in a crucial defensive mechanism through the action of enzymes that use it as a substrates or cofactor. Following the production of oxidised glutathione (GSSG), it conjugates with a number of endogenous and exogenous substances and eliminates superoxide and hydroxyl radicals. GSH also seems to have a role in hydroperoxide-induced oxidative injury's immediate cellular consequences. The free radicals that are essential for antitumor action are directly reduced by GSH. GSH reacts with either hydrophilic or electrophilic substances when GST is present (45). A decrease of peroxides that are present inside the cell is catalysed by GSH-Px. The oxidised GSSG is subsequently converted back into GSH by GR in the presence of NADPH. Thus, the peroxidase/reductase couple's cooperative action mitigates drug-induced oxidative stress (45); as a consequence, greater resistance to anticancer medications is caused by the interaction between GSH and its metabolising enzymes (46). In addition to helping numerous proteins create the proper

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disulfide linkages, glutathione and the enzyme known as glutathione reductase (GR) play a role in the breakdown of xenobiotics (47). A concurrent rise in GSSG and reduction in GSH level is consistent with a decrease of the glutathione reductase activity (48), as glutathione reductase produces GSH from GSSG. According to Saad et al. (40), adriamycin administration also caused peroxidative changes in a number of tissues, which were demonstrated by a substantial decrease in the amount of GSH present in the rat heart, kidney, and liver tissues. In this study, glutathione reductase levels were considerably decreased in HCC-bearing rats by adriamycin treatment, but this was reversed by a combination therapy that included curcumin. The current study demonstrated that, under oxidative stress, the enzyme -GT that utilises GSH is raised while the reducing components of the cell, GSH and its synthesiser -GCS (-glutamyl cysteine synthetase), are diminished. Glutathione synthase and -GCS work together to catalyse the GSH production. Rate limiting factors include cellular cysteine levels and -GCS concentrations. GSH's feed-back suppression of -GCS causes the level of cellular GSH (49,50) to stabilise. When DEN and adriamycin were given to rats, the livers showed reduced -glutamyl cysteine synthetase activity.

GSH controls and the amount of cysteine available limits the feedback inhibition of glutamyl cysteine synthetase activity (51). Adriamycin and curcumin therapy together restored the level of -glutamyl cysteine synthetase to normal. It could be as a result of curcumin's ability to restore glutathione levels that have been depleted by its cytoprotective effects. The NADP/NADPH ratio would change in favour of NADP under oxidative stress, indicating higher glucose-6-phosphate dehydrogenase activity (52) and this claim is supported by data from animals who received DEN and ADR. When used alongside adriamycin, curcumin enhances the body's defence against free radicals, reducing the risk of liver and kidney damage.

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Conclusion:

The chemotherapeutic drug adriamycin is frequently employed in the treatment of cancer. It has demonstrated effectiveness against acute leukaemia, lymphoma, and several other solid human tumours. The therapeutic usage of adriamycin is nevertheless restricted by its documented cardiotoxicity, nephrotoxicity, and hepatotoxicity. The hunt for strategies to reduce adriamycin's harmful effects is still going on. Here, adriamycin and the antioxidant curcumin were used in an effort to reduce their harmful side effects and boost their therapeutic efficiency.

Ethical approval

The study was approved by the Institutional Animal Ethics Committee.

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