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Effects of curcumin on diazinon-induced biochemical and cytogenetical alterations in male rats

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Abstract

The present study was undertaken to pinpoint the possible protective effect of curcumin against subacute toxicity of Diazinon (DZN) induced adverse effect on some biochemical and cytogenetical parameters. 36 male albino rats were classified randomly into six equal groups. Group (G1) was kept as negative control, G2 and G3 were administrated a high and low dose of DZN as 35 and 17.5 mg/kg b.w. over period of 28 days (5 days/week), respectively. G4 received curcumin as 200 mg/kg diet. G5 and G6 were administrated high and low dose of DZN, respectively and co-treated with curcumin. The obtained results revealed that there was a significant increase in liver and kideny function parameters with significant decline in total protein, albumin, beta and gamma globulins together with albumin/globulins ratio (A/G) of G2 and G3 than control. Also, a significant increase in serum tumor necrosis factor-α (TNF-α), liver, kidney and spleen malondialdehyde (MDA), catalase, nitric oxide (NO) with decrease in total antioxidant capacity (TAC) and glutathione (GSH) levels in G2 and G3than control. Moreover, upturn in level of liver and kidney function was observed in G5 and G6. As curcumin is antioxidant and has anti-inflammatory effect could rebalance the state of hypoprotenmia associated with hypoalbunemia, and attenuates the oxidative indices and TNF-α induced by DZN. A significant increase in the percentage of micronucleated polychromatic erythrocytes (MPCE), and significant decrease in normohromatic erythrocytes (NCE) in G2 and G3 than control, whiles the ratio of (PCE/NCE) increase only in G2 than control. On the other hand, co administration of curcumin mixed with diet in case of high and low dose of DZN group's cause a significant decrease in (MPCE), and increase of (NCE) in G5 and G6 in comparing to G2 and G3, while (PCE/NCE) decrease insignificant in G5 and G6 compared to G2 and G3. Histophathological examination revealed degenerative changes in the liver and kidney tissues in G2 and G3. Meanwhile, curcumin alivate this changes in G5 and G6. These results indicated that curcumin may be able to meliorate the worst biochemical and cytogentical alterations caused by high and low doses of diazinon.

Keywords*:* Diazinon –oxidative indices- curcumin -TNF- α-cytogenetic

Introduction

Organophosphates pesticides are commonly used as insecticides. They are generally the most toxic pesticides for animal species especially vertebrate animals **(Shah and Iqbal, 2010)**. Residual amounts of organophosphate insecticides are detectable in soil, vegetables, tissue of organisms, grains, and other food products **(Ogutcu et al., 2006)**. The organophosphorous compounds have a lipophilic nature facilitate their penetration through the cell membrane to induce changes in cell membrane phospholipids, production of free radical of reactive oxygen species and a generation of oxidative stress in different tissues **(West et al., 2013).**

Diazinon (DZN, is a commonly used organophosphorous (OP) pesticide diethoxy- [(2-isoprophyl- 6-methyl-4-pyrimidinyl) oxy]-thioxophosphorane). It is a synthetic chemical substance with broad spectrum insecticide activity **(Sarabia et al., 2009).** The main mechanism of its action is altering normal neurotransmission within the nervous system of target organisms. Non-target organisms can be exposed to diazinon by inhalation, ingestion and/or dermal exposure **(Reigert and Roberts, 1999).** The toxicological effects of DZN in animals and human have been demonstrated in acute and chronic exposures, changes in liver enzymes and biochemical indices and swelling of mitochondria in hepatocytes **(Kalender et al., 2005).**

DZN's mutagenicity studies its ability to cause genetic damage, showed that DZN in fact can damage DNA in human blood cells, in cells from laboratory animals, and in bacteria **(Grover et al., 2003)**. DZN exposure was found to increase the occurrence of a type of genetic damage called micronuclei (MN). Micronuclei may be induced by strand breaks in DNA due to oxidative stress (**Fenech, 1993)**. The MN test using peripheral blood cells is used to detect chromosome breaks that derive from DNA damage **(Igarashi and Shimada, 1997).** Additionally, several studies showed that DZN was capable of inducing histopathological, biochemical and physiological alterations **(Cakici and Akat, 2013; and El-Demerdash and Nasr, 2014).**

Curcumin, (Cur) commonly called diferuloyl methane, is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. Turmeric has been used traditionally for many ailments because of its wide spectrum of pharmacological activities. Curcumin has been identified as the active principle of turmeric **(Aggarwal et al., 2003).** Curcumin has potent antioxidant, anti-inflammatory, antimutagenic and anticarcinogenic properties **(Chainani-Wu, 2003)**. The protective effects of curcumin against chemically-induced hepatotoxicity are well documented and have been attributed to its intrinsic antioxidant properties **(Rukkumani et al., 2004).** Curcumin is considered to be an effective antioxidant against oxidative tissue damage. It can significantly inhibit the generation of reactive oxygen species (ROS) both in vitro and in vivo **(Biswas et al., 2005)**.

TNF-α plays an important role in the regulation of immune cells and the development of systemic inflammation **(Locksley et al., 2001)**. Both *in vitro* and *in vivo* studies showed that curcumin has profound inhibitory effects on the production of TNF-α. In monocytes and alveolar macrophages, curcumin inhibited phorbol myristate acetate (PMA) - or lipopolysaccharide (LPS) - stimulated production of TNF-α **(Abe et al., 1999).** So, the present study was carried out to elucidate the protective effect of curcumin on diazinon subacute toxicity induces some biochemical (liver, kidney function, protein &its fractions, antioxidants and tumer necrosis factor-α) and cytogintical alteration in male albino rats.

Materials and Methods

Materials:

Diazinon, was obtained from ADWIA 60% EC (Emulsifiable concentrate), Cairo, Egypt. The median lethal dose (LD_{50}) of diazinon was determined according to **(Sine, 1990)** and its value was 350 mg/kg b.w. Diazinon was administrated by the stomach tube to rats at 35 and 17.5 mg/kg b.w which represent 1/10 and 1/20 LD_{50}. The emulsion of DZN was diluted by distilled water before use and orally administrated.

Curcumin, obtained from Sigma, St. Louis, Mo, USA. Curcumin was mixed with diet at a dose of 200 mg/kg of diet **(Messarah et al., 2013).**

Animals:

Thirty-six mature Wistar male rats weighing (180-200 g) were purchased from Theodore Bilharz Research Institute, Giza, Egypt. The animals were housed in plastic cages and kept under laboratory conditions with a 12-hour light/dark cycle and a room temperature of 21±3ºC. The animals were provided with food and water *ad libitum* and were acclimatized for two weeks before starting the experiment. The study was approved by the Institutional Animal care and use Committee (IACUC).

Experimental design:

Animals were classified at random into six groups each of six.

1. **Group one**: rats received tap water, fed on basal diet and were considered as the control negative group.
2. **Group two**: rats received 35 mg/kg b.w diazinon (1/10 LD_{50}) over a period of 28 days (5 days /week) **(El-Kashoury and Tag El –Din, 2010)** which represents the high dose.
3. **Group three**: rats received 17.5 mg/kg b.w diazinon (1/20 LD_{50}) over a period of 28 days (5 days /week) which represents the low dose.
4. **Group Four**: rats as postive control, fed on basal diet mixed with curcumin at dose 200 mg/kg diet for 28 days
5. **Group Five**: rats received high dose of diazinon orally (5 days/week) and fed on basal diet mixed with curcumin 200 mg/kg diet for 28 days.

6. **Group six**: rats received low dose of diazinon orally (5 days/week) and fed on basal diet mixed with curcumin 200 mg/kg diet for 28 days.

Collection of samples:

Rats were subjected to diethyl ether anaesthesia on day 29, blood samples were collected from the orbital sinus of each animal by using heparinized capillary tubes into dry clean tube, left to clot, sera were separated and kept at - 40°C for biochemical analysis. Then animals were sacrificed and bone marrow samples were collected from each rat by exposing the femur and evacuating the bone marrow with syringe containing saline for detection cytogentical alteration. Autopsy performed immediately; liver, kidney and spleen tissues were removed and was washed with saline solution, then minced and homogenized (10% w/v) in ice-cold normal saline. The homogenate was centrifuged at 10,000xg for 20 min at 4°C and the resultant supernatant was used for antioxidant assay **(Chitra et al., 1999)**. The second portion of liver and kidney tissues fixed in 10% neutral buffered formalin for histopathological examination.

1. **Biochemical Parameters**

Assessment of the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) according to **Reitman and Frankel (1957)** alkaline phosphatase (ALP) activities according to **Haussament, (1977),** urea according to **Patton and Grouch (1977)** and creatinine according to **Fabiny and Eringhausen (1971)**. Quantitative determination of serum TNF-α concentration was done using (KOMA BIOTECH INC), ELISA Kit and Catalog No. K0331196

Estimation of total protein and electrophoretic pattern were carried out after **Sonnenwirth and Jaret (1980) and Davis (1964),** respectively and calculated according SynGene S. No. 17292*14518 sme*mpcs**.**

Catalase activity; malondialdehyde (MDA) and glutathione (GSH) in tissue were determined according to **Aebi, (1974) Ohkawa et al. (1979)** and **Ellman (1959),** respectively. Total nitric oxide (NO) in tissue contributed by nitrate and nitrite in a system is measured as nitrite after converting all nitrate to nitrite the method described by **(Green et al. (1982) and Nims et al. (1995)** , and total antioxidant capacity (TAC) according to concentrations according to **Koracevic et al. (2001)**.

2. **Cytogencity**

In order to assess the possible mutagenic effect of Diazinon and effect of curcumin, the micronucleus test was performed to detect chromosomal damage associated with the treatment. Micronuclei were identified as dark blue staining bodies in the cytoplasm of the polychromatic erythrocytes (PCEs) according to the protocol mentioned by **Salamon et al. (1980)**. The polychromatic erythrocytes (PCE, 1000 per animal) were screened for micronuclei and the changes in the mitotic activity were assessed on the basis of the ratio of polychromatic to normochromatic erythrocytes (PCE/NCE ratio) **(Hart and Engberg, 1983 and Al- Bekairi et al., 1991**).

3. **Histopathological examination:**

Tissue specimens were fixed in 10% neutral buffered formalin, processed, paraffin embedded and sectioned at 4-6 µ thickness. Tissue sections were stained with Hematoxylin and Eosin stain according to **(Banchroft et al., 1996).**

4. **Statistical analysis:**

Data obtained were statistically analyzed using analysis of variance (ANOVA) using F- test according to **SPSS-21(2012).**

Results and Discussion

Liver is the major site of organophosphorus metabolism so accumulating a great quantity of its metabolites could be possible **(Giray et al., 2001).** As shown in table (1) there was a statistically significant increase in ALT and AST levels in all experimental groups in comparing with the control group, which mostly approached the normal level with curcumin administration. Meanwhile, data showed significant decrease in G5 and G6 in compassion with G2 andG3. Also, there was a significant increase in ALP levels in G2, G3, G5 and G6 in comparison with G1and G4, while, G5 andG6 significantly decreased than G2 and G3. Also, a significant decrease was recorded in G4 in comparison with all experimental groups. These results seem to be in agreement with **Al-Attar (2010) and Messarah et al. (2013)** who showed a significance increase in ALT, AST and ALP levels**.** The elevation of ALT, AST and ALP levels in the current study supported by histophalogical finding in liver which indicated a sever congestion in the central and portal veins as well as sinusoids, Inflammatory cells infiltration was detected in between the degenerated hepatocytes as well as in the portal area **(Fig.1: A, B)**.these result were confirmed by **Al-Attar (2015)** who showed that rats treated with DZN had many severe histopathological alterations including a damage of liver structure along with disarrangement of hepatic strands. Several cells also showed histological features of necrosis. Moreover, an enlargement of the sinusoids and vacuole formations in hepatocytes. However, rats of G5 and G6 were decreased in these indices which is in agreement with **Messarah et al. (2013)** who reported that supplementation of vitamin E and curcumin to DZN-treated rats can alleviate the serum levels of AST, ALT, ALP and LDH, indicating that exogenous antioxidants could protect liver function. The decrease in AST and ALP activities supports the hepatoprotective effects of curcumin, consistent with the findings that curcumin modulated the increased activity of marker enzymes **(Kalpana et al., 2005).** The histopathology results of G5 and G6 corroborated the above findings which indicate a congestion and dilatation were observed in both central and portal veins (**Fig.1: C, D)** but seem to be less in histopathological changes as compared in G2 and G3.

Urea and Creatinine serum levels table (1) were statistically significant increased in G2, G3, G5 and G6 than control group. Also, there was a statiscally significant decrease in urea and creatinine with levels in G5 and G6 in comparison with

G2 and G3. The present study agreed with **Al-Attar and Abu Zeid (2013). Mansour et al. (2005)** reported that kidneys mainly excrete pesticide, and their metabolites. The effect of these compounds on the kidney parenchyma may cause renal dysfunction associated with significant increase in serum urea and creatinine. The current results of histopathological examination of kidney revealed that the highest and the lowest dose of DZN (G2 and G3) had a great disturbance **(Fig 2: A, B)** The cortical portion showed congestion in the blood vessels and glomeruli as well as focal haemorrhage between the degenerated necrosis tubules. Focal haemorrhage was detected also in between the tubules at the corticomedullary portion. Meanwhile, in G6 the tubules showed pyknosis in the nuclei in some while other had karyocytomegally. These findings were similar to the result of **Sarhan and Al-Sahhaf (2011)** who showed that diazinon treatment to rabbits led to degeneration of renal tubules, hypertrophy of glomeruli and leucocytic infiltrations. These results indicated that DZN metabolites caused toxicity in renal system. In accordance, **El-Shenawy et al. (2009)** reported that exposing mice to DZN caused degeneration of renal tubules, atrophy of glomeruli and interstitial inflammatory cells infiltrations. As the immune system plays a vital role against these toxic particles thus it should be boosted to do so. The present study showed significant decrease in serum urea and creatinine in G5 and G6 due to curcumin effect **(Fig 2: C, D).** These result supported by histopathogical changes in kidney that are still better than that of G2 and G3 which showed degeneration in the lining epithelium of some tubules at the cortex with congestion in the glomeruli and blood vessels. Curcumin could reduce functional disturbances in the rat kidney via supporting the kidney against oxidative stress.

TNF-α is a key mediator of the immune and inflammatory responses and controls the expression of the inflammatory gene network. Therefore, the overproduction of TNF-α contributes significantly to the pathological complications observed in many inflammatory diseases **(Kowalski et al., 2001).** Overproduction of proinflammatory cytokines is likely to contribute to manifestation of the systemic inflammatory response, and hence the development of organ failure **(Kotb and Calandra, 2003)**. Looking over table (1) the obtained result revealed that there was a significant increase in the level of serum TNF-α in G2, G3 and G5 in comparison to the control group. Moreover, there was a significant decrease in TNF-α in G4, G5 and G6 in comparison with G2 & G3. The increase TNF-α level coincide with those reported by **Hariri et al. (2010)** that found serum level of TNF-α was increased significantly by diazinon. While**,** TNF-alpha level was significantly decreased in serum level in group treated with crocin**.** Also, **Ahmed et al. (2013)** recorded that the oral administration of diazinon (25 mg/kg) significantly increased brain TNF-α as compared to control group. Co-administration of melatonin and diazinon significantly reduced TNF-α level as compared to diazinon group. Meanwhile, in the present study a significant decrease in TNF-α in G4, G5 and G6 which fed on a diet containing curcumin. Also, was due to its

inhibitory effect on inflammatory mediators **(Gulcubuk et al., 2006). Aggarwal et al. (2013)** stated that curcumin (diferuloylmethane), a component of turmeric (*Curcuma longa*) that is inexpensive, orally bioavailable and highly safe in humans, yet can block TNF-a action and production in *in vitro* models, in animal models and in humans.

The current study revealed that the electrophoretic pattern of serum protein pointed out that diazinon provoked a significant decrease in serum protein concentration with decrease in total globulins and albumins and therefore A/G decreased, as shown in table (2a). Also, the represented data indicate that there is no significant deference in total protein level in comparison between high and low dose of DZN (G2 and G3) also, in comparison between G5 and G6 as in G1 and G4. DZN had alteration effect on total serum protein and subtractions which include significant decrease of α_1, β_1 and γ_1 – globulin whereas a significant increase α_2, β_2 and γ_2 –globulin was evident table (2b). This may be due to effect of diazinon on hepatic cells and immune system. In addition to the significant drop in albumin value and consequently total proteins which might be a result of hepatocellular injury **(Hazarika et al., 2003).** The current study is in agreement with **El Shenaway et al. (2009)** who reported a decrease in albumin and total protein level after two weeks of diazion treatment. Also, in agreement with **Al-Attar (2015)** who found that total protein level was significantly decreased after exposure to DZN for three weeks and indicated that the exposure to DZN caused a severe disturbance of carbohydrates, lipids and proteins metabolism. Also, a decrease in serum total protein in the DZN-treated group may be due to the liver dysfunctions and disturbance in the biosynthesis of protein. **Aly et al. (2010)** mentioned that the Chlorpyrifos-treated animals also exhibited significantly lower total protein and albumin levels than the control animals. Albumin, which is the most abundant blood plasma protein is produced by the liver and several studies have shown that its production can be decreased by OPs such as Chlorpyrifos. Since reductions in albumin levels are generally suggestive of liver disease, it is possible that OPs like Chlorpyrifos alter protein and free amino acid metabolism and their synthesis in the liver **(Abd El-Aziz et al., 2012). Abu Aita et al. (2012)** found a hypoproteinemia associated with hypoalbuminemia in profenofos exposed rats comparable to control group. This may be due to the hepatocellular injury and disturbed amino acid metabolism induced by profenofos. Meanwhile, in the current study the effect of curcumin could appear in inducing a significant increase in G5 and G6 in total protein and electrophoretic pattern as total albumin, globulin in comparing to rats of G2 and G3 of high and low dose of DZN. Similar result by **Abu Aita et al. (2012)** that found that co-administration of propolis with profenofos increase total protein in comparing with treated with profenofos.

The observed hyporoteinemia may also be attributed to the reactive oxygen species (ROS) generated and induced damage to the cellular macromolecules as protein (**Cetin**

et al., 2010). While, curcumin is a potent antioxidant that could modulate protein metabolism through antioxiative action.

Oxidative stress is a consequence of imbalance between the body antioxidant system and pro-oxidant state generated by pesticide toxicity. Endogenous enzymatic and non-enzymatic antioxidants are essential for the conversion of reactive oxygen species to harmless metabolites as well as to protect and restore normal cellular metabolism and functions **(Bebe and Panemangalore, 2003)**. Antioxidants are scavengers that detoxify excessive ROS and have an important role in maintaining oxidant/antioxidant balance in the body **(Agarwal et al., 2012)**

As shown in table (3, 4 and 5) concentrations of MDA, NO levels and catalase activity in liver, kidney and spleen tissues hemogenate have been shown to be significantly increased with lower GSH and TAC concentrations than control levels in animal studies of diazinon exposure in G2 and G3. Meanwhile, a significant decrease was recorded in G5 and G6 in comparing with G2 and G3, respectively in MDA, NO levels and catalase activity whereas a significant increase in GSH and TAC in G5 and G6 in comparing with G2 and G3. It is plausible that impaired oxidant/antioxidant balance can be partially responsible for the toxic effects of diazinon. . These results are correlated with those obtained by **(Mansour et al., 2009** and **Girary et al., 2001)** for liver**; Mohamed et al., 2013)** for kidney and **(Ahmadi et al., 2012)** for spleen. The increased activities of catalase with decrease in total anti-oxidant capacity were due to the presence of diazinon and the production of free radicals, indicating an effective protection against H_2O_2 which is a powerful oxidizing agent and the main precursor of HO **(Teimouri et al., 2006)** in diazinon**.** Meanwhile, administration of curcumin which has a potent antioxidant effect in G5 and G6 could be able to decrease oxidative stress by DZN, and its antioxidant properties seem to mediate such a protective effect, indicated by the reduction of MDA, and CAT as well as the elevation of GSH and TCA levels in different tissues. These in agreement with **Alp et al. (2012)** who found an acute application of malathion (MAL) which induced oxidative stress and increased MDA levels in kidney and liver tissues. Also**,** sulforophane and curcumin, by blocking the toxic effect of malathion, significantly decreased the MDA levels.

Data recorded in table (6) displays the frequencies of micronuleted polychromatic erythrocytes (MPCEs) and the ratio of PCE/NCE in the different experimental groups. DZN caused significant increase of (MPCEs) at both G2 and G3 particularly in high dose (G2) than control group. The account in G5 and G6 was significantly decreased in comparing with G2 and G3. Also, DZN caused significant decrease of (NCE) at both G2 and G3. While, G5 and G6 were significant decreased in comparing with G2 and G3. The PCE/NCE ratio increased significantly in G2 than control group. As regards there is no significance difference between control group and positive control group (G4) in MPCE, NCE and PCE/NCE. Similar results were obtained by **Ahmad et al. (2014)** who found that subcutaneous injection of CCl_4 caused

significant increase in micronucleated polychromatic erythrocytes (MPCEs) and the ratio of polychromatic to normochromatic erythrocytes (PCE / NCE). It caused significant decrease in NCE. Whereas, feeding of curcuma, ginger and rosemary to CCl_4- intoxicated rats caused significant decreases in MPCEs and ratio of PCE/NCE to a value near to normal value. Moreover, **Suzuki et al. (1997)** found that the significant increase in PCE / NCE ratio and enhancement of mitotic activity of bone marrow cells could be considered as a sign of toxicity and / or damage of some organs of the body. **Venees et al. (2011)** reported that DZN is genotoxic as assessed by comet and micronucleus assays which were used for measuring the DNA damage; a significant increase in tail length of comets from blood cells as well in the frequency of micronucleated cells (MNCs) following DZN administration was achieved. Co-administration of vitamin E along with DZN resulted in a decrease in tail length of comets and the percentage of MNCs compared to DZN alone treated rats. The increase in frequency of MNCs and tail length of comets confirm the genotoxicity of DZN. **(Ojha and Srivastava, 2014)** demonstrated genotoxic potential of OP pesticides, **as** they found a Significant increases in strand breaks and in levels of the reactive oxygen species (ROS) superoxide anion and hydrogen peroxide were observed in lymphocytes treated with pesticides. Methyl parathion (MPT) exposure caused the greatest DNA damage and ROS production, followed by chlorpyrifos (CPF) and malathion (ML). Furthermore, organophosphate toxicity may be attributted in part to the generation of reactive oxygen species and reactive oxygen free radicals can damage DNA through oxidation of DNA bases or through covalent binding to DNA resulting in strand breaks and cross linking **(Saulsbury et al., 2009).**

Conclusion

This study indicated that curcumin is able to ameliorate the toxic effect of diazinon (subacute toxicity) on some biochemical and cytogenic indices. As curcumin has potent antioxidant, anti-inflammatory and antimutagenic effects and could be used as protective agent from chemically-induced oxidative stress.

Table (1): The effect of diazinon, curcumin and their combination on liver, kideny functions and tumor necrosis factor-α

Parameter / Groups	**ALT** u/l	**AST** u/l	**ALP** u/l	**Urea** mg/dl	**Creatinine** mg/dl	**TNF-α** pg/ml
Control (G1)	24.17^{e} ±1.07	38.17^{f} ±1.28	141.67^{e} ±1.05	40.58^{d} ±1.00	0.57^{f} ±0.06	155.17^{d} ±1.2
DZN(1/10 LD_{50}) (G2)	70.75^{a} ±1.70	89.17^{a} ±1.42	249.50^{a} ±3.33	76.83^{a} ±1.28	1.38^{a} ±0.05	394.67^{a} ±2.78
DZN(1/20 LD_{50}) (G3)	58.88^{b} ±1.95	67.83^{b} ±1.70	196.83^{b} ±1.85	59.83^{b} ±1.47	1.12^{b} ±0.06	209.67^{b} ±4.44
Cur (G4)	31.00^{d} ±1.41	43.58^{e} ±2.00	134.67^{f} ±1.45	44.00^{d} ±1.37	0.45^{e} ±0.04	145.0^{e} ±2.22
DZN(1/10 LD_{50}) +Cur (G5)	56.67^{b} ±1.75	62.67^{c} ±1.38	188.83^{c} ±1.32	51.83^{b} ±1.08	1.05^{c} ±0.06	175.08^{c} ±2.18
DZN(1/20 LD_{50}) +Cur (G6)	45.67^{c} ±1.58	49.00^{d} ±1.65	167.17^{d} ±1.97	47.50^{c} ±0.72	0.87^{d} ±0.03	160.42^{d} ±1.83

Data are expressed as means ± SE, (n=6).

Means in the same column with different superscripts are significantly different ($P \leq 0.05$).

DZN: Diazinon, **Cur:** Curcumin, **TNF-α:** Tumor necrosis factor-α.

Table (2a): The effect of diazinon, curcumin and combination of both on total and main fractions of serum protein in male rats

Parameters / Groups	Total Protein (g/dL)	Pre Alb (g/dL)	Alb (g/dL)	Total Alb (g/dL)	Total Alpha (g/dL)	Total Beta (g/dL)	Total Gamma (g/dL)	Total Globulin (g/dL)	A/ G Ratio
Control (G1)	6.49±0.12 [a]	0.37±0.01 [a]	1.41±0.03 [ab]	1.78±0.05 [a]	1.63±0.09[b]	1.57±0.05[b]	1.53±0.03 [a]	4.71±0.08 [a]	0.38±0.003 [a]
DZN (1/10 LD_{50}) (G2)	5.33±0.03 [c]	0.16±0.02 [d]	1.16±0.02 [d]	1.31±0.04 [d]	1.77±0.03 [a]	1.44±0.04[c]	0.81±0.01 [c]	4.02±0.04 [b]	0.33±0.011 [b]
DZN (1/20 LD_{50})(G3)	5.59±0.18 [c]	0.21±0.02 [cd]	1.25±0.03 [cd]	1.46±0.04 [c]	1.72±0.03 [a]	1.52±0.04[b]	0.89±0.13 [bc]	4.13±0.17 [b]	0.36±0.012[ab]
Cur (G4)	6.59±0.16 [a]	0.36±0.003 [a]	1.43±0.06 [a]	1.78±0.06 [a]	1.63±0.06 [a]	1.49±0.04[c]	1.68±0.05 [a]	4.80±0.11 [a]	0.37±0.01[a]
DZN (1/10 LD_{50}) +Cur (G5)	6.11±0.04 [b]	0.27±0.03[bc]	1.31±0.08[bc]	1.57±0.03[bc]	1.73±0.005 [a]	1.72±0.04 [a]	1.08±0.02[b]	4.54±0.06 [a]	0.35±0.011[ab]
DZN (1/20 LD_{50}) + Cur (G6)	6.24±0.06 [ab]	0.29± 0.01[b]	1.35±0.02[b]	1.64±0.03[b]	1.59±0.02[b]	1.53±0.02[b]	1.48±0.07 [a]	4.59±0.08 [a]	0.36±0.012[ab]

Data are expressed as means ± SE, (n=6).
Means in the same column with different superscripts are significantly different (P≤ 0.05).
DZN: Diazinon, **Cur:** Curcumin.

Table (2b): The effect of diazinon, curcumin and combination of both on subtractions of serum protein in male rats

Parameters / Groups	Alpha 1 (g/dL)	Alpha 2 (g/dL)	Beta 1 (g/dL)	Beta 2 (g/dL)	Gamma 1 (g/dL)	Gamma 2 (g/dL)
Control (G1)	0.39±0.01 [a]	1.21±0.09[b]	0.76±0.03[b]	0.82±0.02[b]	1.08±0.01 [a]	0.45±0.02[d]
DZN(1/10 LD_{50}) (G2)	0.21±0.02 [d]	1.56±0.02 [a]	0.50±0.01[d]	0.97±0.03 [a]	0.037±0.02[c]	0.77±0.02 [a]
DZN(1/20 LD_{50}) (G3)	0.26±0.01 [c]	1.47±0.02 [a]	0.63±0.02[c]	0.89±0.03 [a]	0.23±0.12[d]	0.65±0.03[b]
Cur (G4)	0.35±0.04 [ab]	1.27±0.04[b]	0.73±0.03[b]	0.76±0.01[c]	1.17±0.03 [a]	0.51±0.03[c]
DZN(1/10 LD_{50})+ Cur (G5)	0.25±0.01 [c]	1.48±0.02 [a]	0.84±0.03 [a]	0.88±0.03 [a]	0.54±0.03[b]	0.54±0.02[c]
DZN(1/20 LD_{50})+ Cur (G6)	0.30±0.01[bc]	1.29±0.03[b]	0.69±0.01[c]	0.83±0.02[b]	0.97±0.05 [a]	0.51±0.02[c]

Data are expressed as means ± SE, (n=6).
Means in the same column with different superscripts are significantly different (P≤ 0.05). **DZN:** Diazinon, **Cur:** Curcumi

Table (3): Effect of diazinon and curcumin their and combination on some antioxidants parameter of liver in male rats

Parameters / Groups	MDA m.mol/g Tissue	Catalase n.mol/g Tissue	NO m.mol/g Tissue	TAC n.mol/g Tissue	GSH m.mol/g Tissue
Control (G1)	1.64±0.1 ^{d}	0.32 ±0.01 ^{d}	0.51±0.03 ^{d}	6.12±0.06 ^{b}	5.00±0.34 ^{b}
DZN(1/10 LD_{50}) (G2)	5.65±0.14 ^{a}	0.64 ±0.02 ^{a}	0.77±0.02 ^{a}	3.65±0.18 ^{e}	2.36±0.13 ^{d}
DZN(1/20 LD_{50}) (G3)	3.85±0.38 ^{b}	0.53 ±0.03 ^{b}	0.69±0.02 ^{b}	4.38±0.08 ^{d}	2.63±0.02 ^{d}
Cur (G4)	1.51±0.1 ^{d}	0.34 ±0.02 ^{d}	0.51±0.02 ^{d}	6.75±0.05 ^{a}	5.95±0.11 ^{a}
DZN(1/10 LD_{50})+ Cur (G3)	4.11±0.05 ^{b}	0.54 ±0.01 ^{b}	0.62±0.02 ^{c}	5.52±0.08 ^{c}	3.42±0.34 ^{c}
DZN(1/20 LD_{50})+ Cur (G6)	3.16±0.04 ^{c}	0.43 ±0.01 ^{c}	0.55±0.01 ^{d}	6.17±0.01 ^{b}	3.68±0.30 ^{c}

Data are expressed as means ± SE, (n=6).

Means in the same column with different superscripts are significantly different (P≤ 0.05)

DZN: Diazinon, **Cur:** Curcumin, **MDA**: Malondialdehyde, **NO:** Nitic oxide, **TAC**: Total antioxidant capacity, **GSH** Gulthione.

Table (4): Effect of diazinon and curcumin and their combination on some antioxidants parameter of kidney in male rats

Parameters / Groups	MDA m.mol/g Tissue	Catalase n.mol/g Tissue	NO m.mol/g Tissue	TAC n.mol/g Tissue	GSH m.mol/g Tissue
Control (G1)	0.72±0.17 ^{c}	0.29±0.02 ^{de}	0.77±0.05 ^{c}	5.74±0.06 ^{a}	4.83±0.23 ^{b}
DZN(1/10 LD_{50}) (G2)	2.71±0.11 ^{a}	0.68±0.07 ^{a}	1.62±0.03 ^{a}	3.08±0.13 ^{e}	1.33±0.12 ^{e}
DZN(1/20 LD_{50}) (G3)	1.81±0.14 ^{b}	0.54±0.02 ^{b}	1.29±0.01 ^{b}	3.93±0.08 ^{d}	2.38±0.09 ^{d}
Cur (G4)	0.42±0.08 ^{c}	0.20±0.01 ^{e}	0.67±0.02 ^{d}	5.90±0.04 ^{a}	6.25±0.013 ^{a}
DZN(1/10 LD_{50})+ Cur (G5)	2.03±0.12 ^{b}	0.45±0.03 ^{bc}	0.86±0.01 ^{c}	4.57±0.03 ^{c}	2.73±0.09 ^{d}
DZN(1/20 LD_{50})+ Cur (G6)	1.86±0.08 ^{b}	0.37±0.01 ^{cd}	0.81±0.03 ^{c}	5.33±0.05 ^{b}	3.63±0.13 ^{c}

Data are expressed as means ± SE, (n=6).

Means in the same column with different superscripts are significantly different (P≤ 0.05).

DZN: Diazinon, **Cur:** Curcumin, **MDA**: Malondialdehyde, **NO:** Nitic oxide, **TAC**: Total antioxidant capacity, **GSH** Gulthione.

Table (5): Effect of diazinon and curcumin and their combination on some antioxidants parameter of spleen in male rats

Parameters / Groups	MDA m.mol/g Tissue	Catalase n.mol/g Tissue	NO m.mol/g Tissue	TAC n.mol/g Tissue	GSH m.mol/g Tissue
Control (G1)	1.02±0.03^{e}	0.60±0.04 ^{d}	0.60±0.05 ^{c}	6.27±0.02 ^{a}	3.89±0.08^{a}
DZN(1/10 LD_{50}) (G2)	3.83±0.2^{a}	1.38±0.07 ^{a}	0.80±0.01 ^{a}	4.15±0.07 ^{e}	1.04±0.01^{d}
DZN(1/20 LD_{50}) (G3)	2.45±0.09^{c}	1.20±0.04 ^{b}	0.74±0.01^{b}	5.03±0.04 ^{d}	1.84±0.09^{c}
Cur (G4)	0.88±0.07^{e}	0.61±0.03 ^{d}	0.39±0.01 ^{d}	6.09±0.10 ^{b}	4.05±0.24^{a}
DZN(1/10 LD_{50})+ Cur (G5)	3.30±0.05^{b}	0.86±0.03 ^{c}	0.71±0.01 ^{b}	5.15±0.07 ^{d}	2.93±0.11^{b}
DZN(1/20 LD_{50})+ Cur (G6)	1.82±0.1^{d}	0.65±0.02 ^{a}	0.68±0.01 ^{c}	5.70±0.04 ^{c}	2.90±0.10^{b}

Data are expressed as means ± SE, (n = 6).

Means in the same column with different superscripts are significantly different (P≤ 0.05).

DZN: Diazinon, **Cur:** Curcumin, **MDA**: Malondialdehyde, **NO:** Nitic oxide, **TAC**: Total antioxidant capacity, **GSH** Gulthione

Table (6): Effect of diazinon, curcumin and their combination on percentage of MPCE and PCE/NCE ratio in male rats

Parameters / Groups	PCE	MPCE/1000 PCE	NCE	PCE/NCE
Control (G1)	5000	5.6 ±0.081^{e}	2183 ±31.89^{a}	2.290 ±0.42^{b}
DZN(1/10 LD_{50}) (G2)	5000	15.64±0.25^{a}	740.9±16.518^{e}	6.796 ±0.276^{a}
DZN(1/20 LD_{50}) (G3)	5000	8.63 ±0.53^{c}	1507.6±19.76^{c}	3.316 ±0.29^{b}
Cur (G4)	5000	5.43 ±0.06^{e}	2180.31±31.05^{a}	2.293±0.08^{b}
DZN(1/10 LD_{50})+ Cur (G5)	5000	11.437±0.36^{b}	952.0 ±5.2^{d}	5.25±0.36^{a}
DZN(1/20 LD_{50})+ Cur (G6)	5000	7.1±0.46^{d}	1694.02 ±7.01^{b}	2.95±0.07^{b}

Data are expressed as means ± SE, (n=6).

Means in the same column with different superscripts are significantly different (P≤ 0.05)**.**

DZN: Diazinon, **Cur:** Curcumin, **MPCE:** micronuleted polychromatic erythrocytes,

PCE: poly chromatic erythsrocytes **,** **NCE:** normochromatic erythrocytes.

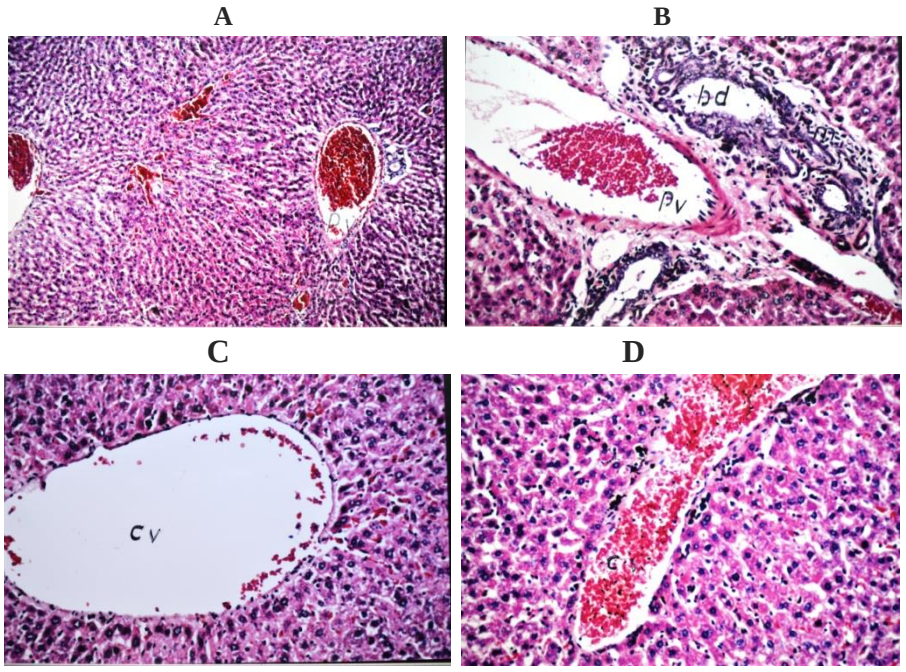

Fig 1: (A): Liver of rats in G2 showing the sever congestion in portal vein (P.V)**, (B):** Liver of rats in G3 showing the inflammatory cells infiltration in portal area, **(C):** Liver of rats in G5 showing the dialated central vein (C.V)**, (D):** Liver of rats in G6 showing the congested central vein (C.V). H&E, x40

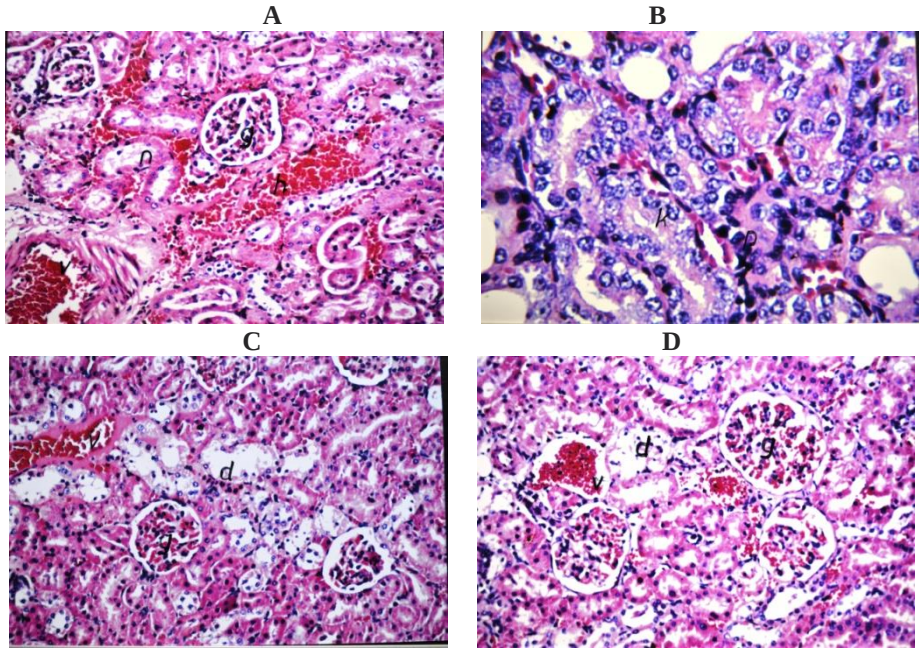

Fig 2: (A): Kidney of rats in G2 showing congestion in blood vesssels (V) and glomeurli (g) with focal haemorage (h) between the degenerated and necrosed tubules (n). H&E, x40**, (B):** Kidney of rats in G3 showing pyknosis in nuclei of some tubules (p) with karyocytomegaly in lying tubular epithelium. H&E, x80**, (C):** Kidney of rats in G5 showing degeneration in lying epithelium (d) of some tubules H&E, x40**, (D):** Kidney of rats in G6 showing degeneration in lying epithelium of some tubules (D) with congestion in glomeurli (g) and blood vessels (v) H&E, x40

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