



## Haematological parameters in rats treated with royal jelly and curcumin antioxidants after CCL4haemotoxicity.

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### ABSTRAT

The aim of the present work is to explore the possible effect of royal jelly and curcumin on CCL4 intoxicated albino rats. Concerning haematological parameters (RBCs count ,WBCs count , Hb content , HCT value and blood indices ( MCV,MCH and MCHC ) values ,lymphocytes percentage and monocytes percentage in rats injected with CCL4 for four and six weeks . The results referred to a significant decrease in RBCs count , Hb content , HCT , MCV , MCH values and lymphocytes percentage in rats injected with CCL4 at four and six weeks. While rats treated with oral administration of royal jelly and curcumin after CCL4 injection recorded improvements in red blood cells and hemoglobin content. On the other hand a significant increase in WBCs count and monocytes percentage were recorded in rats injected with CCL4 at four and six weeks.

**Key Words:** Royal jelly; Curcumin; Rats; CCL4; blood parameters.



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## 1. INTRODUCTION

Carbon tetrachloride (CCL4) is a halo alkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, particularly in refrigerator fluids, as a dry cleaning agent in industry, as a household spot remover. As a result of its wide spread use, CCL4 is a common contaminant of ground and surface waters where it persists for years. Therefore CCL4 is now of greatest concern as an environmental contaminant. [1].

CCL4 is one of the chlorinated hydrocarbons that have a wide spread use in various industries as a solvent and also use in medicine as vermifuge in treatment of hookworm disease. Prolonged administration of CCL4 causes increase in concentration of serum hepatic enzymes and also leads to fibrosis, cirrhosis and hepatic carcinoma. [2].

Carbon tetrachloride (CCL4) is an industrial solvent and xenobiotic used to induce chemical hepatitis injuries in laboratory animals. The CCL4 induced hepatitis lesions are a common experimental model for the screening of the hepatoprotective activity of certain drugs. [3,4] found a significant decrease in hemoglobin concentration after administration of CCL4 for seven days. Also [5] found that the treatment with CCL4 for 45 days significantly decreased the red blood cells count, white blood cells count, haematocrit value and hemoglobin level.

Injection of rats with CCL4 caused a significant reduction in RBCs count, HCT value, Hb content and WBCs count. [6,7]. Also the same authors observed that oral administration of curcumin before CCL4 injection reduced the suppressive effect of CCL4 on RBCs count, HCT value, Hb content and WBCs count.

A significant decrease in RBCs count, HCT value and Hb concentration after injection with CCL4 (1mg/kg) three times weekly for two weeks. Administration of CCL4 produced a significant increase in the number of total leucocytes and polymorphs in blood. [8,9].

Curcumin (Cur), a yellow pigment obtainable from the rhizomes of the plant *Curcuma longa*, is a crystalline compound that has been traditionally used in medicine and culinary practices in India. Recently, curcumin was found to possess chemo preventive effect against skin cancer, stomach cancer, colon cancer and oral cancer in mice. It possesses various pharmacological effects via antioxidant, anti-inflammatory, anti-thrombotic and hepatoprotective. [10, 11] Curcumin is a bioactive food component present in the golden spice *Curcuma longa*, also curcumin modulates the biological activity of many signaling molecules. It has anti cancer activities that have been demonstrated in many studies which attributed to its antioxidant and anti-inflammatory properties. [12,13].

Royal jelly exerted the significant protective effect on liver damage as well as on oxidative stress induced by CCL4, resulting in reduced lipid peroxidation and improved endogenous antioxidant defense systems. [14], Royal jelly has been demonstrated to possess many pharmacological activities in experimental animals, including antitumor, antioxidant, anti-inflammatory, antibacterial and antihypertensive properties. [15].

## 2. MATERIALS AND METHODS

### 2.1. Experimental design.

The present study was carried out on 80 male albino rats average weight between (180-200 g). The rats were kept in clean cages. Food and water were provided *ad libitum*. The rats were randomly divided into eight groups as follow:-

The first group: control group. The second group: Rats were injected intraperitoneal with CCL4 in dose of (2ml/kg) diluted in olive oil 1: 1 twice weekly for four and six weeks. The third group: Rats were given orally royal jelly in dose of (500 mg/ kg b .w) dissolved in distal water and administered daily for four and six weeks. The fourth group: Rats were given orally curcumin in dose of (150 mg/ kg b .w) dissolved in olive oil and administered daily for four and six weeks. The fifth group : Rats were given orally curcumin and royal jelly in doses of (150 mg/ kg and 500 mg/ kg b .w ) and administered daily for four and six weeks.. The sixth group: Rats were injected intraperitoneal with CCL4 and administered royal jelly for four and six weeks. The seventh group: Rats were injected intraperitoneal with CCL4 and administered curcumin for four and six weeks. The eighth group: Rats were injected intraperitoneal with CCL4 and administered curcumin and royal jelly for four and six weeks. Each group contains ten rats, at the end of fourth week five rats from each group were scarified and the remained five rats were scarified after six weeks for taken blood samples.

### 2.2. Hematological parameters.

Blood samples were collected from the jugular vein of the rats in avial containing ethylene diamine tetra acetic acid (EDTA). Erythrocytes (RBCs) count, hemoglobin (Hb) concentration, haematocrit value (HCT) value, leucocytes (WBCs) count, lymphocytes percentage and monocytes percentage was estimated by blood cell counter (CBC instrument SK 9000).

### 2.3. Statistical analysis.

Statistical analysis of the obtained data was done according to [16], using T-test value.

## 3. RESULTS

As summarized in table 1 results indicated a significant decrease (  $P < 0.05$  and  $P < 0.01$ ) in red blood cells (RBCs) count, hemoglobin (Hb) content, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in rats injected with CCL4 for four weeks. On the other hand insignificant difference in RBCs count, Hb content, HCT, MCV



and MCHC values was observed in the remained groups, except groups received CCL4 with royal jelly, CCL4 with curcumin, CCL4 with royal jelly and curcumin showed a significant decrease ( $P < 0.05$  and  $P < 0.01$ ) in MCV and MCH values when compared with the control rats.

Data found in table 2 demonstrated that rats received CCL4 for six weeks recorded a significant decrease ( $P < 0.05$  and  $P < 0.01$ ) in RBCs count, Hb content, HCT and MCV values as compared with the control group. Also groups received CCL4 with royal jelly and CCL4 with curcumin showed a significant decrease ( $P < 0.01$ ) in HCT and MCV values, also a significant decrease ( $P < 0.01$ ) in MCV value was observed in rats received CCL4 with royal jelly and curcumin. Hemoglobin content revealed a significant decrease ( $P < 0.05$ ) in rats received CCL4 with curcumin, while insignificant difference in RBCs count, Hb content, HCT, MCV, MCH and MCHC values were showed in the remained groups in comparison with the control.

Results demonstrated in table 3 refer to a significant increase ( $P < 0.05$  and  $P < 0.01$ ) in white blood cells (WBCs) count, and monocytes percentage in rats received CCL4, CCL4 with royal jelly, CCL4 with curcumin and CCL4 with royal jelly and curcumin for four weeks in comparison with the control rats. In contrast, a significant decrease ( $P < 0.01$ ) in lymphocytes percentage in rats received CCL4, CCL4 with royal jelly, CCL4 with curcumin and CCL4 with royal jelly and curcumin for four weeks, while insignificant difference was observed in WBCs count, monocytes percentage and lymphocytes percentage in the remained groups in comparison with the control rats.

Data represented in table 4 showed a significant increase ( $P < 0.01$ ) in white blood cells (WBCs) count in rats received CCL4 for six weeks, also a significant increase ( $P < 0.01$ ) in monocytes percentage was observed in rats received CCL4, CCL4 with royal jelly, CCL4 with curcumin and CCL4 with royal jelly and curcumin for six weeks in comparison with the control rats. On the other hand a significant decrease ( $P < 0.01$ ) in lymphocytes percentage in rats received CCL4, CCL4 with royal jelly, CCL4 with curcumin and CCL4 with royal jelly and curcumin for six weeks, while insignificant difference was recorded in WBCs count, monocytes percentage and lymphocytes percentage in the remained groups in comparison with the control rats.

**Table1: Haematological parameters (RBCs, Hb, HCT, MCV, MCH and MCHC) in rats treated with royal Jelly and curcumin after injection with CCL4 for four weeks.**

Groups parameters		Control	CCI4 (2mg/kg)	Royal jelly (500mg/kg)	Curcumin (150mg/kg)	Curcumin + royal jelly	CCI4+ royal jelly	CCI4+ curcumin	CCI4+ royal jelly + curcumin
RBCs x 10 <sup>6</sup> , Cell/mm <sup>3</sup>	Mean ± S.E Prob.	5.75 ± .35 —	4.88 ± 0.11 P < 0.05	5.82 ± 0.09 N.S	5.96 ± 0.08 N.S	5.84 ± 0.15 N.S	6.09 ± 0.31 N.S	5.68 ± 0.12 N.S	6.02 ± 0.14 N.S
Hb, g/dl	Mean ± S.E Prob.	14.42 ± 0.51 —	12.26 ± 0.21 P < 0.01	14.32 ± 0.35 N.S	14.62 ± 0.26 N.S	14.12 ± 0.51 N.S	14.86 ± 0.58 N.S	13.64 ± 0.24 N.S	14.52 ± 0.39 N.S
HCT, %	Mean ± S.E Prob.	37.08 ± 0.49 —	34.72 ± 0.99 N.S	37.94 ± 0.88 N.S	37.8 ± 0.46 N.S	36.4 ± 1.54 N.S	38.24 ± 0.71 N.S	36.24 ± 0.25 N.S	36.98 ± 0.37 N.S
MCV, μ <sup>3</sup>	Mean ± S.E Prob.	67.68 ± .67 —	60.88 ± 0.58 P < 0.01	64.68 ± 1.94 N.S	65.86 ± 0.51 N.S	65.66 ± 0.87 N.S	56.60 ± 0.49 P < 0.01	58.84 ± 0.91 P < 0.01	57.12 ± 0.58 P < 0.01
MCH, Pg	Mean ± S.E Prob.	25.96 ± .38 —	24.0 ± 0.42 P < 0.01	25.0 ± 0.64 N.S	25.32 ± 0.30 N.S	25.70 ± 0.31 N.S	24.64 ± 0.37 P < 0.05	24.10 ± 0.32 P < 0.01	23.58 ± 0.30 P < 0.01
MCHC, g%	Mean ± S.E Prob.	39.38 ± .77 —	37.72 ± 1.10 N.S	38.06 ± 0.46 N.S	38.24 ± 0.35 N.S	39.26 ± 0.48 N.S	39.82 ± 0.55 N.S	40.64 ± 0.29 N.S	40.28 ± 0.47 N.S

- Prob. = Probability
- N.S. = Non significant
- P < 0.05 = Significant (\*)
- P < 0.01 = Highly significant (\*\*)



**Table2: Haematological parameters (RBCs, Hb , HCT, MCV, MCH and MCHC) in rats treated with royal Jelly and curcumin after injection with CCL4 for six weeks.**

Groups parameters		Control	CCI4 ( 2mg/kg )	Royal jelly (500mg/kg )	Curcumin (150mg/kg )	Curcumin + royal jelly	CCI4+ royal jelly	CCI4+ curcumin	CCI4+ royal jelly + curcumin
RBCs x 10 <sup>6</sup> , Cell/mm <sup>3</sup>	Mean ± S.E Prob.	5.87 ±0.11 —	4.69 <sup>*</sup> ± 0.22 P < 0.01	5.95 ±0.12 N.S	5.56 ± 0.13 N.S	5.55 ± 0.15 N.S	5.82 ±0.19 N.S	5.63 ±0.21 N.S	6.09 ±0.19 N.S
Hb, g/dl	Mean ± S.E Prob.	14.22 ±0.43 —	12.14 ± 0.41 P < 0.01	13.68 ±0.32 N.S	13.36 ± 0.22 N.S	13.24 ± 0.22 N.S	12.74 ± 0.49 N.S	12.54 ± 0.32 P < 0.05	13.98 ± 0.32 N.S
HCT, %	Mean ± S.E Prob.	36.86 ± 0.60 —	30.37 ± 1.91 P < 0.05	36.08 ± 0.69 N.S	35.16 ± 0.59 N.S	35.43 ± 0.27 N.S	31.48 ± 0.98 P < 0.01	32.20 ± 0.66 P < 0.01	37.46 ± 0.92 N.S
MCV, μ <sup>3</sup>	Mean ± S.E Prob.	66.56 ± 0.83 —	60.28 ± 0.86 P < 0.01	64.40 ± 0.61 N.S	68.58 ± 1.65 N.S	65.54 ± 0.39 N.S	55.08 ± 0.59 P < 0.01	57.32 ± 0.39 P < 0.01	57.40 ± 0.45 P < 0.01
MCH, Pg	Mean ± S.E Prob.	24.54 ± 0.97 —	23.68 ± 0.46 N.S	22.90 ± 0.11 N.S	26.0 ± 0.79 N.S	25.46 ± 0.45 N.S	22.26 ± 0.33 N.S	22.42 ± 0.22 N.S	22.78 ± 0.32 N.S
MCHC, g%	Mean ± S.E Prob.	38.72 ± 0.85 —	38.12 ± 0.79 N.S	37.26 ± 0.34 N.S	37.60 ± 0.46 N.S	38.34 ± 0.41 N.S	39.70 ± 0.56 N.S	38.52 ± 0.34 N.S	37.46 ± 0.35 N.S

Prob. = Probability  
 N.S. = Non significant  
 P < 0.05 = significant (\*)  
 P < 0.01 = Highly significant (\*\*)





**Table3: Total leucocytes (WBCs) count, lymphocytes percentage and monocytes percentage in rats treated with royal jelly and curcumin after injection with CCL4 for four weeks.**

Groups parameters		Control	CCI4 ( 2mg/kg )	Royal jelly (500mg/kg )	Curcumin (150mg/kg )	Curcumin + royal jelly	CCI4+ royal jelly	CCI4+ curcumin	CCI4+ royal jelly + curcumin
WBCs x 10 <sup>3</sup> , Cell/mm <sup>3</sup>	Mean	12.28	16.54	13.08	14.18	12.58	15.46	14.78	14.26
	± S.E	±0.43	± 0.52	± 0.76	± 1.12	± 0.47	± 0.34	± 0.35	± 0.50
	Prob.	—	P < 0.01	N.S	N.S	N.S	P <0 .01	P < 0.01	P < 0.05
lymphocytes, %	Mean	75.82	49.98	72.06	73.96	67.76	60.48	57.96	63.78
	± S.E	± 1.61	± 3.58	± 1.18	± 1.97	± 4.85	± 0.74	± 3.06	± 0.71
	Prob.	—	P < 0.01	N.S	N.S	N.S	P <0 .01	P < 0.01	P < 0.01
Monocytes, %	Mean	7.34	13.22	7.45	7.96	8.12	13.1	13.14	13.36
	± S.E	±0.15	± 1.32	± 0.26	± 0.43	± 0.71	± 0.47	± 0.38	± 0.46
	Prob.	—	P < 0.01	N.S	N.S	N.S	P < 0.01	P < 0.01	P < 0.01

Prob. = Probability  
 N.S. = Non significant  
 P < 0.05 = significant (\*)  
 P < 0.01 = Highly significant (\*\*)

**Table4: Total leucocytes (WBCs) count, lymphocytes percentage and monocytes percentage in rats treated with royal jelly and curcumin after injection with CCL4 for six weeks.**

Groups parameters		Control	CCI4 ( 2mg/kg )	Royal jelly (500mg/kg )	Curcumin (150mg/kg )	Curcumin + royal jelly	CCI4+ royal jelly	CCI4+ curcumin	CCI4+ royal jelly+ curcumin
WBCs x 10 <sup>3</sup> , Cell/mm <sup>3</sup>	Mean	11.82	22.22	11.56	10.68	10.64`	11.04	10.19	11.68
	± S.E	±0.45	± 1.37	± 0.24	± 0.39	± 0.48	± .50	± 0.78	± 0.36
	Prob.	—	P < 0.01	N.S	N.S	N.S	N.S	N.S	N.S
lymphocytes, %	Mean	74.26	46.62	72.88	71.00	72.90	59.98	59.80	57.76
	± S.E	±0.94	± 4.19	± 1.47	± 3.25	± 0.33	±2.22	± 0.36	± 2.78.
	Prob.	—	P < 0.01	N.S	N.S	N.S	P < 0.01	P < 0.01	P < 0.01
Monocytes, %	Mean	7.68	12.46	9.25	9.12	7.94	11.3	13.38	12.22
	± S.E	±0.22	± 0.72	± 0.76	± 0.91	± 0.37	± 0.33	± 0.78	± 0.92
	Prob.	—	P < 0.01	N.S	N.S	N.S	P < 0.01	P < 0.01	P < 0.01

Prob. = Probability  
 N.S. = Non significant  
 P < 0.05 = significant (\*)  
 P < 0.01 = Highly significant (\*\*)



#### 4. DISCUSSION

Intraperitoneal injection of CCL4 greatly affected all hematological parameters, as decrease in RBCs count, Hb content and HCT, value, this decrease maybe due to internal hemorrhaging as a result of decreased synthesis of clotting factors by the liver or direct effect on bone marrow cells or maybe destruction of red blood cells. [17,18,19] state that the reduction in blood values of blood parameters (RBCs, Hb and HCT,) may be attributed to the hyper activity of bone marrow, which leads to production of RBCs with impaired integrity that are easily destroyed in the circulation. Elevation in white blood cells count are observed, perhaps in response to necrosis in the liver or kidneys. [20].

Alterations in RBC- membrane and the loss of functional integrity of the membrane precede the onset of CCL4 induced liver cirrhosis. erythrocytes are at high risk from damage by reactive oxygen species due to direct exposure of the extracellular fluid to chemical assaults. [21] The depression in RBCs count and hemoglobin content could be attributed to disturbed hematopoiesis, destruction of erythrocytes and reduction in the rate of their formation. [22] The administration of CCL4 induced macrocytic hypochromic anemia. [23]. Mention that curcumin has been shown in the last two decades to be a potent immune modulator agent. The significant decrease in RBCs count, Hb, HCT, and WBCs count could be attributed to CCL4 toxicity. The features of anemia disappeared in the treated groups with curcumin and royal jelly and the blood values returned nearly to the normal levels, this is in accordance with [24] who stated that curcumin as an active ingredient of turmeric and anti inflammatory agent could disrupt interactions between circulating blood cells and endothelium and improve its survival.

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