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Hemato-Biochemical Alterations Mediated by Carbendazim Exposure and Protective Effect of Quercetin in Male Rats

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Abstract

The main objective of study was to investigate the effect of Carbendazim (CBZ) a most popular agriculture fungicide on hemato-biochemical alterations in male rats and possible ameliorative effect of quercetin. Sprague Dawley male rats were administered CBZ @100, 200 and 400 mg/kg body weight, *PO* and quercetin (25mg/kg body weight, *PO*) alone and concomitant in corn oil for the period of 28 days. At the end of exposure period, blood was collected and hemato-biochemical parameters were evaluated in blood and plasma samples. Sub-acute exposure of carbendazim resulted in non-significant decrease ($p > 0.05$) in haemoglobin, PVC and TEC and increase in MCV, MCH and platelets count in CBZ treated groups. Moreover, administration of CBZ significantly increased ($p < 0.05$) the plasma ALT, AST, ALP, cholesterol and triglyceride while, significantly decreased ($p < 0.05$) the plasma protein and globulin level was noticed. Non-significant change in calcium, chloride and phosphorous was observed in CBZ alone treated groups. However, quercetin supplementation concomitant in CBZ treated animals prevented and restored the alterations caused by exposure of CBZ. This study concluded that quercetin has ability to ameliorate the toxic effect caused by CBZ on hemato-biochemical biomarkers in living beings.

Keywords: Carbendazim, Haemato-Biochemical, Quercetin, Rats

1. Introduction

The use of synthetic pesticides in agro-vet practices started in 1950 and has been increased many fold in last two decade due to intensification of farming in order to fulfill the demand of consumers. Currently, India is the largest producer of pesticides in Asia and even consumer in the world. Although pesticides as well as fungicides plays a key role in plant disease control and subsequent increase in the yield and crop productivity. However, there have been reports of several ill effects on health such as neurotoxicity, embryotoxicity, hepatotoxicity, testicular toxicity, immunotoxicity, nephrotoxicity, genotoxicity, carcinogenicity[1-5]. Car-Benda-Zim (CBZ) (methyl-2-benzimidazole carbamate) is one of the broad spectrum most widely used fungicide in agriculture sector. It primary acts on fungal cell tubulins, causing disruption of microtubules formation and inhibition of mitotic cell division[4,5]. CBZ is used as a preservative in paint, textile, papermaking and leather industry as well

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as a preservative for fruits[6,7], thus it is one of the most widely used, broad spectrum fungicides for its application in current years. Moreover, future consequences have been reported by many researchers such as effect on hypothalamic, pituitary and other endocrine glands, thus it is categorized under endocrine disrupter pesticides. It has deleterious effect on testis, sexual maturity, development of foetus and hormonal regulation in mammalian system[8,9] and thereby it may induce male infertility or testicular toxicity[10,11]. A long-term exposure to CBZ is also associated with thyroid gland dysfunction and hepato-nephrotoxicity[12,13]. Several *in vivo* as well as *ex vivo* studies suggest that CBZ may causes oxidative stress and it has harmful effect on biochemical and hematological indices[8,14].

The alternation in some of the hematological and biochemical parameters might reflect an early stage in the development of a disease mediated by many environmental toxicants. In recent years, more attention has been given for the assessment of alteration in hemato-biochemical indicators associated with pesticides exposure in animals and humans. Moreover, literature survey also indicates that pesticide-induced oxidative stresses also considered as a possible mechanism of toxicity and hence focus of toxicological research diverted towards it. The exposure of animals or human beings to pesticides, heavy metals or xenobiotics in the body increases free radical generation[15,16]. Free radicals attack on essential proteins which are oxidized resulting into diminution of sulfhydryls or thiols contents, DNA which is damaged via free radicals mediated oxidative reactions, cellular lipid membrane which rapidly react with free radicals and undergo peroxidation (LPO) and finally leading to cell damage, change in blood cell components in the living systems. Ultimately, these molecular changes caused by toxicants may be measured in terms of hematological and biochemicals alternation as a first line toxic mechanism[17].

Further, the natural medicaments might be helpful in ameliorating hematological and biochemical alterations induced by environmental toxicants. Evidence showed that consumption of natural antioxidants lead to a decrease in various pro-inflammatory and/or oxidative stress biomarkers and activates the efflux pump[18]. A report pertaining to the ameliorative effect of antioxidants on carbendazim has been reported by many workers. Selenium supplementation partially protected from testicular oxidative injury caused by carbendazim in bucks has been[9,14]. Protective effect of licorice aqueous extract, kolaviron (seed of *Garcinia kola*) and vitamin E on carbendazim-induced testicular toxicity in animal has been reported[10,19]. Further, protective effects of 6-Gingerol-rich fraction from ginger and Nigella sativa oil on hematotoxicity, hepatorenal damage and genotoxicity caused by carbendazim has been reported[12,13].

Quercetin (3,5,7,3',4'-pentahydroxyflavone), a natural plant bioflavonoid, is a strong antioxidant and radical scavenger which present abundant in fruits (strawberry, apple etc.), leafy vegetables, beverages (tea, red wine, beer etc.) and onions. Quercetin has been reported for its anti-inflammatory, antioxidant, anti-blood coagulant, anti-ischemic effects, and anti-matrix metalloproteinase action[20]. Scientific studies indicates that quercetin has potential to protective against toxic effect caused by scopolamine[21], cadmium[22] and, haloperidol[23]. Quercetin is also found to be protective in oxidative neuronal injuries and, diabetic rat retina[24]. In present article, an endeavour has been made to find out degree of toxic manifestation of CBZ on hemato-biochemical biomarkers and, ability of quercetin in minimizing the toxic effect of CBZ in mammalian systems.

2. Materials and Methods

2.1 Chemicals and Reagents

Technical grade carbendazim (97%) and quercetin (≥95 %) was procured from Sigma Aldrich, USA. All the chemicals of analytical grade and enzymes used in the present study were obtained from established firms such as Bangalore-Genei, Himedia, Merck, and Span Diagnostics Pvt. Ltd. Buffers and reagents were prepared in autoclaved Milli-Q water and filtered through membrane filters (0.22 µm) purchased from Millipore (Molsheim, France).

2.2 Experimental Condition

Prior to start of experiment, necessary approval was obtained from the Institutional Animal Ethics Committee of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, India (Approval no. GADVASU/2016/IAEC/33/11; dated: 20.01.2016). All experiment has been carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. Sub-acute toxicity study was carried out in 54 number adult male Sprague Dawley rats which were procured from National Institute of Pharmaceutical Education and

Research (NIPER), Mohali, Punjab, India. Rats were caged in the polyacrylic cages in the departmental animal house. The animals were maintained under standard managemental conditions. The animals were provided with standard rat feed (Ashirwad industries, Mohali Punjab) and water *ad libitum*. Room temperature was maintained around 22 ± 3°C throughout the experiment with 12 hour of light and 12 hour of dark cycle.

2.3 Experimental Design

The animals were randomly divided into nine groups comprise of six animals in each. CBZ and Quercetin were suspended in corn oil and administered by oral gavage for the period of 28 days.

1. Group I: Rats provided with normal feed and deionized water serve as control.
2. Group II: Rats treated with corn oil (1ml/kg body weight) serve as vehicle control.
3. Group III: Rats treated with CBZ-I (100 mg/kg body weight daily for 28 days).
4. Group IV: Rats treated with CBZ-I (200 mg/kg body weight daily for 28 days).
5. Group V: Rats treated with CBZ-I (400 mg/kg body weight daily for 28 days).
6. Group VI: Rats treated with Quercetin (25 mg/kg body weight daily for 28 days).
7. Group VII: Rats treated with CBZ-I plus Quercetin identically as in groups III and VI.
8. Group VIII: Rats treated with CBZ-II plus Quercetin identically as in groups IV and VI.
9. Group IX: Rats treated with CBZ-III plus Quercetin identically as in groups V and VI.

2.4 Observations Recorded

All animals were observed twice daily for appearance of any change in gross observable behavioural signs and symptoms of toxicity like water intake, feed intake, diarrhoea, dyspnoea, hyperactivity, tremor, convulsions and death if any.

At the end of experiment experimental, 24 hrs after the last dose, blood samples were collected directly from heart in heparinized container from all the rats. A measured volume of blood about 1 ml was taken for haematological studies and rest of the blood was used for separation of plasma. Plasma was separated out and stored at -20°C for various enzymes, and biochemical estimations.

Various haematological parameters were analyzed using hemoanalyzer (Model: EM 200, Erba®, Mannheim, USA) *viz.*:

1. Haemoglobin (Hb): The results were expressed as g/dl of blood.
2. Packed Cell Volume (PCV): The results were expressed in %.
3. Total Leucocyte Count (TLC): The results were expressed in $\times 10^3$ /µl.
4. Total Erythrocyte Count (TEC): The results were expressed as $\times 10^6$ /µl.
5. Differential Leucocyte Count (DLC): The results were expressed in cells/ µl.
6. Mean Corpuscular Volume (MCV): The results were expressed in femtolitre (Fl)
7. Mean Corpuscular Haemoglobin (MCH): The results were expressed in picogram (pg)
8. Mean Corpuscular Haemoglobin Concentration (MCHC): The results were expressed in %.

The sera samples were analyzed for parameters such as ALanine amino-Transferase (ALT), Aspartate amino-Transferase (AST) and cholesterol using standard kits from Biozyme Diagnostics Kalher, Thane. Alkaline Phosphatase (ALP), triglyceride, Lactate De-Hydrogenase (LDH), calcium (Ca) and chloride (Cl) were measured using kits obtained from Coral Clinical Systems, Goa, India. Albumin and phosphorus (P) was estimated with Erba Diagnostics Mannheim, Germany and total protein was estimated using standard commercial kits from Autopak Siemens.

2.5 Statistical Analysis

Statistical analysis of data was carried out by employing one way ANOVA with Duncan multiple comparison as post hoc test and the p value of < 0.05 was considered as statistically significant. Data was analyzed by using IBM SPSS, Version 20.0 statistical software and Microsoft Excel. All values were expressed as Mean ± SEM.

3. Results

Repeated administration of CBZ and Quercetin, alone and in combination did not produce any mortality and signs of toxicity in rats. Slight change in consistency of faeces and rough body coat was noticed in high dose of

CBZ treated group rats at the end of experiment. Average daily feed (Figure 1) and water intake (Figure 2) was decrease in CBZ treated groups compared to control group. Whereas, quercetin supplementation resulted in increase in feed and water intake in all CBZ-quercetin treated groups as compared to their corresponding CBZ alone treated groups. Apparently less weight gains were noticed in high dose CBZ alone treated group compared to control and CBZ plus quercetin treated groups (Table 1).

Effect was noticed on TLC, PCV, MCV, MCH and, platelet count. Quercetin administration resulted in significant increase ($p < 0.05$) in TLC, PCV, MCV, MCH and, platelet count in CBZ treated groups (Table 2A & 2B). Significant ($p < 0.05$) decrease in percent lymphocytes and eosinophils was noticed in CBZ alone treated groups as compared to control group. However, simultaneous administration of quercetin along with CBZ showed significant ($p < 0.05$) increase in lymphocyte and eosinophils count (Table 2C).

CBZ treatment resulted in significant increase ($p < 0.05$) in the level of ALT, AST and ALP as compared to control groups. Significant decrease ($p < 0.05$) in ALT, AST and ALP level was noticed in CBZ-quercetin co-treated animals. Electrolytes values of plasma Ca, P and Cl were non-significantly ($p > 0.05$) increased in CBZ alone treated groups, whereas Ca, P and Cl plasma levels were decreased in CBZ-quercetin co-exposed groups (Table 3A).

Plasma protein levels were significantly decreased ($p < 0.05$) in CBZ alone treated groups in dose dependent manner as compared to control group, while, significantly increased ($p < 0.05$) in CBZ-quercetin co-treated groups as compared to the CBZ alone treated groups (Table 3A). CBZ exposure resulted in significant increase ($p < 0.05$) in concentration of cholesterol, triglyceride, albumin and, albumin: globulin (A:G) ratio and, decrease in globulin concentration when compared with control group. Whereas, quercetin administration in CBZ treated rats resulted in significant decrease ($p < 0.05$) in cholesterol, triglyceride, and albumin and A: G ratio with increase in globulin concentration as compared to the respective CBZ alone treated rats (Table 3B).

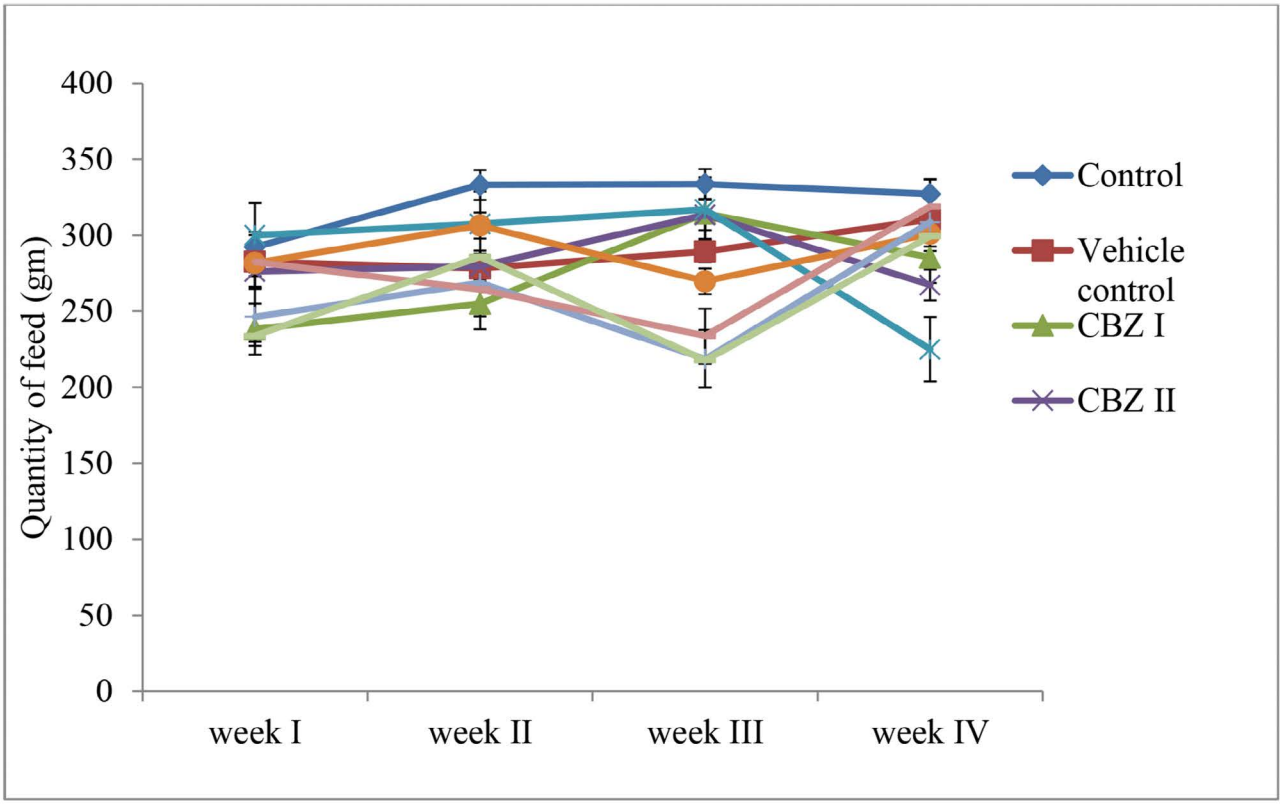


Figure 1. Effect on average feed intake per group on per day basis (gm) when rats exposed to CBZ, quercetin and their combination (n = 6).

4. Discussion

The weight gain in rats and other animal species serve as a positive physiological indicator for increase in growth rate and good health whereas decrease in body weight, body weight gain and relative organ weight is used to study pathophysiological state of animals under experimental conditions which may indicate toxicity[25]. Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals[26,27]. Decrease body weight and weight gain was observed in CBZ alone treated groups may be due to decrease in feed intake. Less feed intake due to loss of appetite could be responsible for the reduced body weight gain in CBZ treated groups. There was gradient decrease in feed and water intake in CBZ alone treated groups was observed in present study. This may be indicative of CBZ induced toxicity hampering the basal metabolism of the body and subsequently resulting into decreased feed and water intake. Similarly, loss in feed consumption was detected in rats and mice, which had received high doses of triadimefon, CBZ and acetamiprid[28,29].

Improvement in body weight in all quercetin incorporated groups in this study may be attributed to improvement in the metabolic process, slightly restore feed intake as compared to CBZ alone treated groups. This also indicates that quercetin has protected the animals from the toxic effect of CBZ on metabolic organ, gastrointestinal epithelial cells and oxidative stress. It also indicates the adaptation of mammalian system against the CBZ induced adverse effect and, counteracted the toxicity being induced by CBZ and, consequently improvement in feed intake and body weight gain. The ameliorative potential of different antioxidants on food consumption against toxicants has been supported by many researchers, such as; vitamin E was assessed against malathion[30], broccoli extract against triazophos[31] and quercetin against thiamethoxam[32] in different species of animals. In all the cases the body weights were significantly improved. However, quercetin co-treatment in CBZ treated animals restored the relative weight gain.

Hematological parameters are generally assessed to determine the functional health status and the internal environment of an organism[33,34]. A haematological

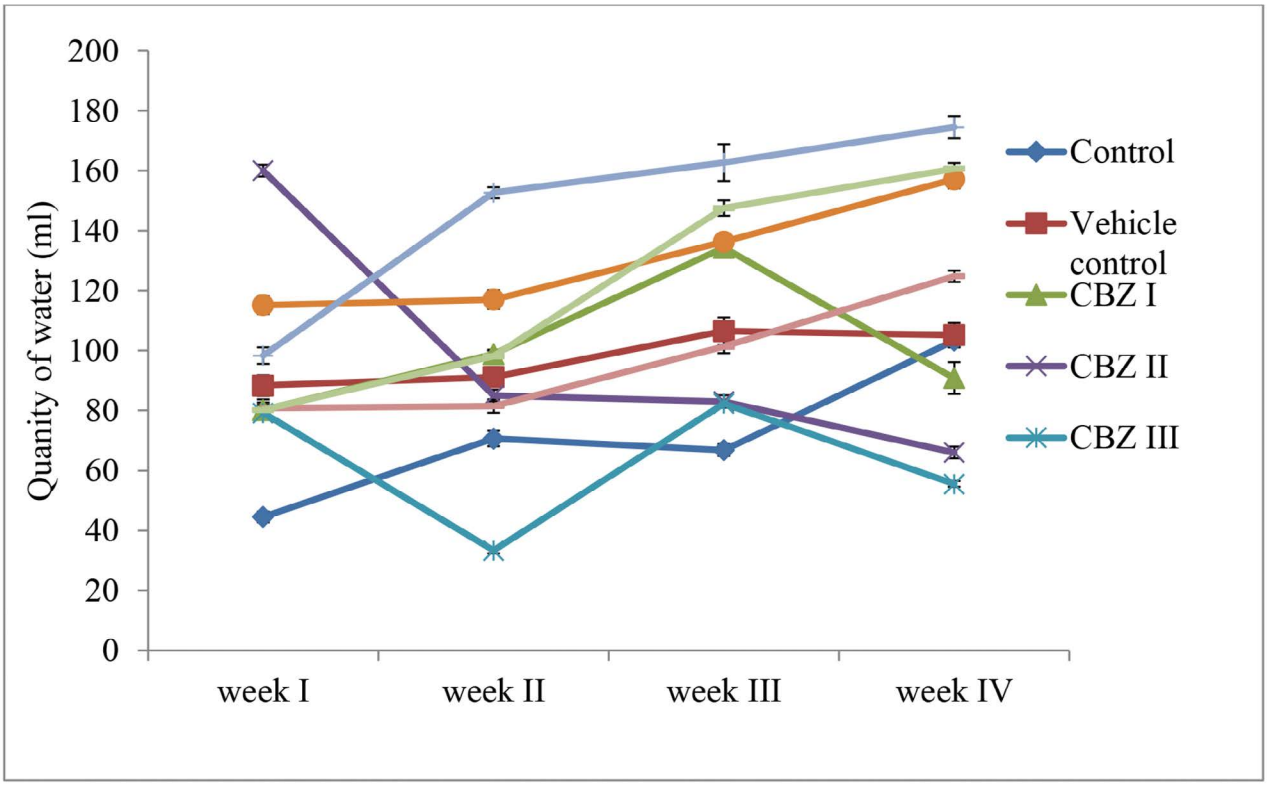


Figure 2. Effect on average water intake per group on per day basis (ml) when rats exposed to CBZ, quercetin and their combination (n = 6).

Table 1. Effect on body weight gain (g) and percent body weight gain (%) on sub-acute exposure to carbendazim, quercetin and their combination *per os* in male rats

Groups	0 day	28 day	B.wt. gain	% wt. gain
I	269.17 ± 10.36^{a}	297.50 ± 8.16^{a}	28.33 ± 4.21^{ab}	9.07 ± 1.51^{ab}
II	257.50 ± 11.31^{a}	297.17 ± 9.77^{a}	39.67 ± 10.16^{a}	13.37± 3.69^{a}
III	279.33 ± 6.36^{a}	298.67 ± 6.6^{a}	19.33 ± 7.28^{ab}	5.99 ± 2.28^{ab}
IV	264.33 ± 11.13^{a}	289.67 ± 7.94^{a}	29.33 ± 6.17^{ab}	9.67 ± 2.32^{ab}
V	268.33 ± 3.33^{a}	274.67 ± 4.16^{a}	16.33 ± 5.23^{b}	5.19 ± 1.66^{b}
VI	275.17 ± 8.96^{a}	303.83 ± 9.57^{a}	28.67 ± 6.43^{ab}	8.92 ± 1.98^{ab}
VII	275.33 ± 8.59^{a}	302.83 ± 9.49^{a}	27.50 ± 3.79^{ab}	8.49 ± 1.19^{ab}
VIII	251.33 ± 18.51^{a}	283.83 ± 15.89^{a}	32.50 ± 9.24^{ab}	11.56± 3.89^{ab}
IX	260.33 ± 8.67^{a}	295.17 ± 8.13^{a}	34.83 ± 3.03^{ab}	11.32± 1.18^{ab}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

Table 2A. Effect of CBZ, QRC and their combination on haematological parameters at end of exposure period

Groups	PCV (%)	Hb (g/dl)	WBC (x10^{3}/ µl)	TEC (x10^{6}/µl)	Platelets (x10^{3}/µl)
I	58.08 ± 0.98^{a}	16.75 ± 0.28^{ab}	8.55 ± 0.21^{d}	9.76 ± 0.05^{a}	348.67 ± 3.18^{f}
II	55.90 ± 1.6^{abc}	16.30 ± 0.3^{abc}	8.59 ± 0.05^{d}	9.73 ± 0.07^{ab}	376.83 ± 5.76^{e}
III	56.00 ± 0.67^{abc}	16.17 ± 0.29^{abc}	3.44 ± 0.35^{bcd}	9.49 ± 0.11^{ab}	429.33 ± 5.91^{bc}
IV	53.07 ± 0.88^{c}	16.05 ± 0.25^{abc}	3.10 ± 0.36^{cd}	8.83 ± 0.23^{c}	411.17 ± 5.13^{d}
V	53.37 ± 0.58^{c}	16.38 ± 0.2^{abc}	5.68 ± 0.05^{b}	9.70 ± 0.04^{ab}	415.67 ± 4.01^{cd}
VI	57.13 ± 1.58^{ab}	17.00 ± 0.32^{a}	8.26 ± 0.72^{a}	9.78 ± 0.14^{a}	446.83 ± 4.21^{a}
VII	52.55 ± 1.63^{c}	15.65 ± 0.51^{c}	8.30 ± 1.95^{a}	9.28 ± 0.24^{b}	439.50 ± 5.66^{ab}
VIII	54.78 ± 0.33^{abc}	16.28 ± 0.09^{abc}	5.48 ± 0.31^{bc}	9.58 ± 0.12^{ab}	419.17 ± 5.17^{cd}
IX	53.98 ± 0.82^{bc}	15.85 ± 0.28^{bc}	5.4 ± 0.81^{bc}	9.45 ± 0.14^{ab}	422.67 ± 2.85^{cd}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

CBZ treatment did not produced any significant effect ($p > 0.05$) on Hb and TEC, while, significant ($p < 0.05$) . A haematological analysis indicates that, CBZ has dose-dependent decrease in RBC, WBC and lymphocyte counts. Decrease in the red blood cells indicates that CBZ disrupt the erythropoiesis process or an increase in destruction of red blood cells[34], increase in the premature RBC in circulation and, impaired Hb biosynthesis[35]. It has been reported that a reduction in the RBCs, Hb, PCV, MCHC, and neutrophils with an elevation in MCV, WBC count, lymphocytes, and eosinophils responsible for macrocytic hypochromic anemia when animal exposed individually or co-exposed to carbendazim and mancozeb[13]. The reduction in white blood cell and lymphocyte counts may show that CBZ has an immune-suppressive effect on mammalian system. Lymphocytes are one of the important cells of the immune system. In addition, CBZ-treated rats showed an increase in MCH, MCV as well as MCHC values suggest that CBZ might have negative effect on other blood-forming organs. These findings are in accordance with previous studies, which indicated that treatment of rats with CBZ caused decrease in RBC count, Hb concentration and PCV values[6,36] and even in benomyl, a parent compound of CBZ. Decrease in PCV value is also attributed to the reduction in RBC count caused by either destruction or reduction in size. Further, decrease in the WBC count and is directly related to the decreased in production from the germinal center of

lymphoid organs or increased in lysis of WBC due to the presence CBZ or toxicants in the body system[37].

CBZ is a dicyclic compound having imidazole and benzene fused ring. It has been suggested that compounds having benzene ring or other structural similarity act as a hapten that combines with the protein constituent of leukocytes to form an antigen, to which the animal develops antibodies and that are toxic to leukocytes, causing either lysis or agglutination. These results are consistent with the report showing decrease in total leukocyte count, when imidacloprid (a benzene ring-structured compound) a member of neonicotinoid pesticide, when it was administered to male White Leghorn chicks[38]. An increase of erythrocyte size (MCV) has been associated with several factors but it is generally considered as a response to stress exerts by CBZ or toxicants and have toxic effect on hematological parameters[39].

ALT and AST leaked into the circulation indicating hepatocellular injury in response to toxicants. The increase in plasma ALP, ALT and AST level indicates the altered function of liver and kidney due to CBZ exposure in present study. These findings are in agreement with previous reports that repeated oral administration of CBZ significantly elevated plasma ALT, AST, ALP, and GGT level in rats co-administered with 6-GRF and CBZ. This increase in the plasma levels indicates hepatic and renal function[12]. Similarly, increased in level of LDH, AST, ALT, GGT, ALP, creatinine and albumin with use of pesticides, CBZ in male goat has been reported[8]. It has also been noticed that CBZ exposure increased the maternal levels of cholesterol, triglyceride and creatinine in female mice[40]. However, slight change in cholesterol may also be an indication of altered carbohydrate and fat metabolism. Similarly, several investigations showed that the values of blood triglycerides and cholesterol were evoked in CBZ and other pesticides-treated animals[36,41]. A number of different hepatotoxic agents also cause accumulation of fatty deposits, predominantly triglycerides in

Table 2B. Effect of CBZ, QRC and their combination on haematological parameters at end of the exposure period

Groups	MCV (fl)	MCH(pg)	MCHC(g/dl)
I	59.52 ± 1.07^{ab}	17.16 ± 0.31^{b}	28.85 ± 0.43^{b}
II	57.53 ± 1.99^{abc}	16.76 ± 0.34^{b}	29.24 ± 0.72^{ab}
III	59.08 ± 0.96^{ab}	17.05 ± 0.34^{b}	28.90 ± 0.71^{b}
IV	60.25 ± 1.43^{a}	18.24 ± 0.57^{a}	30.25 ± 0.28^{ab}
V	55.02 ± 0.55^{c}	16.89 ± 0.23^{b}	30.71 ± 0.32^{a}
VI	58.39 ± 1.14^{abc}	17.39 ± 0.25^{ab}	29.83 ± 0.72^{ab}
VII	56.63 ± 0.59^{bc}	16.87 ± 0.26^{b}	29.78 ± 0.2^{ab}
VIII	57.19 ± 0.52^{abc}	17.01 ± 0.25^{b}	29.73 ± 0.18^{ab}
IX	57.14 ± 0.55^{abc}	16.78 ± 0.22^{b}	29.36 ± 0.29^{ab}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

Table 2C. Effect of CBZ, QRC and their combination on differential leucocyte count at end of the exposure period

Groups	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Monocytes (%)
I	84.33 ± 1.23^{a}	15.00 ± 0.52^{bc}	2.50 ± 0.43^{a}	4.33 ± 0.21^{ab}
II	84.17 ± 0.54^{a}	16.17 ± 0.3^{ab}	2.33 ± 0.33^{ab}	3.67 ± 0.21^{b}
III	82.67 ± 0.61^{abc}	15.00 ± 0.26^{bc}	2.17 ± 0.31^{abc}	4.83 ± 0.31^{a}
IV	81.50 ± 0.43^{bcd}	16.00 ± 0.26^{ab}	1.67 ± 0.21^{abcd}	4.67 ± 0.17^{ab}
V	79.83 ± 0.54^{d}	17.00 ± 0.37^{a}	1.17 ± 0.17^{d}	4.85 ± 0.31^{a}
VI	84.33 ± 1.23^{a}	15.00 ± 0.52^{b}c	1.50 ± 0.22^{bcd}	2.67 ± 0.21^{c}
VII	83.5 ± 0.56^{ab}	14.00 ± 0.26^{c}	1.67 ± 0.21^{abcd}	3.67 ± 0.21^{b}
VIII	83.67 ± 0.33^{ab}	15.00 ± 0.26^{bc}	2.17 ± 0.17^{abc}	2.67 ± 0.21^{c}
IX	81.00 ± 0.52^{cd}	16.00 ± 0.37^{ab}	1.33 ± 0.21^{cd}	2.50 ± 0.22^{c}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

Table 3A. Effect of CBZ, QRC and their combination on biochemical parameters at end of the exposure period

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Protein (mg/dl)
I	39.07 ± 2.11^{ef}	123.04 ± 3.33^{de}	220.44 ± 3.37^{de}	58.69 ± 1.33^{g}	55.39 ± 2.86^{d}	7.45 ± 0.12^{c}
II	40.81 ± 1.34^{e}	120.56 ± 3.11^{e}	222.25 ± 5.52^{de}	60.99 ± 0.38^{g}	54.32 ± 2.78^{d}	7.52 ± 0.12^{c}
III	55.49 ± 1.4^{c}	136.47 ± 2.08^{c}	247.83 ± 4.79^{bc}	73.32 ± 0.79^{e}	78.00 ± 1.73^{a}	6.48 ± 0.02^{d}
IV	61.53 ± 2.33^{b}	157.59 ± 4.36^{b}	265.09 ± 5.54^{b}	84.29 ± 0.89^{c}	80.00 ± 1.75^{a}	6.87 ± 0.25^{d}
V	70.40 ± 1.37^{a}	173.15 ± 3.36^{a}	317.76 ± 6.67^{a}	93.23 ± 1.11^{a}	82.00 ± 2.60^{a}	5.59 ± 0.04^{e}
VI	35.16 ± 0.92^{f}	122.90 ± 2.75^{de}	216.44 ± 3.44^{e}	61.46 ± 0.9^{g}	60.52 ± 2.11^{c}	8.42 ± 0.13^{b}
VII	46.58 ± 1.85^{d}	130.09 ± 2.49^{cd}	239.57 ± 8.11^{cd}	66.98 ± 0.46^{f}	72.89 ± 1.25^{bc}	7.53 ± 0.08^{c}
VIII	54.22 ± 1.07^{c}	151.26 ± 1.96^{b}	255.80 ± 9.17^{bc}	78.17 ± 0.73^{d}	76.00 ± 1.49^{bc}	8.38 ± 0.09^{b}
IX	63.89 ± 1.46^{b}	171.50 ± 2.91^{a}	310.76 ± 8.8^{5a}	89.67 ± 0.55^{b}	79.00 ± 1.40^{ab}	9.11 ± 0.23^{a}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

Table 3B. Effect of CBZ, QRC and their combination on biochemical parameters at end of the exposure period.

Groups	Albumin (mg/dl)	Globulin (mg/dl)	A:G Ratio	Calcium (nmol/L)	Phosphorous (nmol/L)	Chloride (nmol/L)
I	2.67 ± 0.01^{cd}	4.78 ± 0.12^{ab}	0.56 ± 0.01^{b}	9.77 ± 0.89^{a}	8.51 ± 0.1^{cd}	91.78 ± 1.9^{a}
II	2.78 ± 0.01^{cd}	4.74 ± 0.13^{ab}	0.59 ± 0.02^{b}	9.89 ± 1.13^{a}	8.16 ± 0.16^{d}	92.91 ± 2.09^{a}
III	3.45 ± 0.02^{bcd}	3.03 ± 0.04^{cd}	1.14 ± 0.02^{b}	10.25 ± 1.91^{a}	10.50 ± 0.08^{abc}	96.64 ± 0.84^{a}
IV	4.19 ± 0.02^{abc}	2.68 ± 0.27^{d}	1.64 ± 0.16^{a}	10.43 ± 1.14^{a}	11.74 ± 0.14^{a}	101.99 ± 1.38^{a}
V	5.50 ± 0.04^{a}	0.09 ± 0.04^{e}	0.98 ± 0.15^{b}	11.92 ± 0.9^{a}	12.74 ± 0.14^{a}	105.15 ± 0.6^{a}
VI	2.65 ± 0.02^{cd}	5.77 ± 0.13^{a}	0.46 ± 0.01^{b}	9.13 ± 0.86^{a}	7.20 ± 0.16^{d}	92.07 ± 0.64^{a}
VII	2.44 ± 0.08^{d}	5.09 ± 0.15^{ab}	0.48 ± 0.03^{b}	9.41 ± 0.54^{a}	9.24 ± 0.27^{bcd}	100.27 ± 0.86^{a}
VIII	3.32 ± 0.06^{bcd}	5.06 ± 0.09^{ab}	0.66 ± 0.02^{b}	9.63 ± 0.59^{a}	10.91 ± 0.02^{ab}	96.56 ± 1.28^{a}
IX	4.72 ± 0.01^{ab}	4.39 ± 0.23^{bc}	1.09 ± 0.06^{b}	10.57 ± 0.28^{a}	11.32 ± 0.19^{a}	105.55 ± 2.5^{a}

Values (Mean ± SEM, n=6) bearing different superscripts in the same column differ significantly ($p < 0.05$) in Duncan multiple comparison post hoc test.

the parenchyma cells in the liver. This accumulation of triglycerides may be as a result of an imbalance between the rate of synthesis and the rate of release of triglycerides by the parenchyma cells into the systemic circulation[42]. Additionally, increased in cholesterol level in serum due to liver and kidney damage by CBZ exposure in rats and decreased with an olive oil leaves extract has been reported[36].

Slightly increase in level of plasma Ca, P and Cl ion in CBZ alone treated groups were noticed in this study. These elements have been known to exert pharmacological effects in body[43]. Macro elements such as sodium, chloride, phosphorous, potassium and calcium regulate the fluid balance of the body and play important role in several enzymatic reactions as co-factors. Calcium ion regulates a number of important physiological and biochemical processes in the body. Increase and decrease in plasma sodium and chloride levels may affect the release of hormones and neurotransmitters synthesis, storage and metabolism[44-45]. Altered level of Ca and P

level in present study might be due to altered enzymatic activity of ALP which has direct relation in mineralization in bony matrix. Another possibility is that CBZ might be interfering directly in mineralization process or calcification in bony matrix and may caused demineralization. Elevated P level in this study may be due to damage to phospholipids membrane and DNA damage responsible cumulative accumulation in the circulation. Altered function of kidney, liver and brain responsible for utilization of these ions for cellular function and nerve transmission further aggravate their concentration in body fluid.

Antioxidants have the capacity to scavenge free radical when present in abundance, can significantly contribute to overall ROS scavenging activity by directly or/else interfere with the generation and provide the primary defense against cytotoxic oxygen or free radicals. Quercetin counteracts the potential lethal oxidative stress caused by Quinone Reductase 2 (QR2) in malarial parasites and promote the action quinine has been reported[46]. Quercetin reduced visceral adipose tissue TNF-α and nitric oxide production and downregulated nitric oxide synthase expression[47]. Quercetin increased the plasma concentration of adiponectin, reduced NOx levels in plasma, and lowered VAT TNF- α production in obese Zucker rats[47]. Dietary quercetin suppressed western diet induced gene expression associated oxidative stress and NFκB activity[48], blockes IL-12 signalling and Th1 differentiation[49], inhibites LPS-induced expression of TNF-α, IL-1β and IL-6 by suppressing activation of ERK and p38 MAP kinases in macrophages contributed to the anti-inflammatory and immunostimulant activity. Quercetin induces significant gene expression and production of Th-1-derived interferon (IFN)-g, as well as downregulating Th-2-derived IL-4 production by normal peripheral blood mononuclear cells[47]. The anti-inflammatory profile of quercetin has impact on the recruitment of immune cells and preventing from development of secondary infections following disruption of the skin barrier[50].

Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes and membrane proteins often crucial for a cellular specific function[51]. Quercetin inhibits the production and release of histamine and other allergic and inflammatory substances, possibly by stabilizing cell membranes of mast cells[52] Quercetin is an inhibitor of human mast cell activation through the inhibition of Ca^{2+} influx, histamine, leukotrienes and prostaglandins release and protein kinase activation[25]. Mast cells are influential immune cells important for the pathogenesis of allergic responses and autoimmune disorders. They affect release of many cytokines involved in the inflammatory reactions such as IL-8 and TNF[53].

Quercetin has structure, 2,3 double bound in conjunction with 4-oxo bond in the C ring of quercetin, allows electron delocalization from the B ring and shows extensive resonance. This results in the significant efficiency for radical scavenging. Quercetin seems to be one of the most powerful flavonoids for protecting the body against reactive oxygen species[54]. Quercetin, similar to fisetin, catechin, and myricetin, are flavonoid aglycones that have 3-OH groups and are potent inhibitors of lipid oxidation[55]. Quercetin inhibits the initiation step in chain oxidation and prevents chain propagation. This may also include the termination of a chain by the reaction of two radicals[50,56]. This unique mechanism of action could be responsible for the providing protection against various environmental toxicants and CBZ, who responsible for hematological and biochemical alterations.

The exposure of CBZ resulted in haemotoxicity and altered biochemical parameters. However, quercetin was found to be effective in ameliorating CBZ mediated haemotoxicity and normalized the levels of biochemical parameters. Finding of present study can be concluded that quercetin has ability to ameliorate the toxic effect caused by CBZ on hemato-biochemical biomarkers in animals.

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6. Conflict of Interest

There are no conflicts of interest.

7. References

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