

Full Length Research Paper

Evaluation of curcumin toxicity in rats through biochemical and hematological parameters

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Curcumin is a natural compound known for hepatic and nephroprotective actions, however, there is little research related to its possible side effects. The research aimed to study hematological, biochemical parameters and histopathology of hepatic and renal tissues to evaluate the effects of curcumin toxicity in male rats. A group of Wistar male rats (n = 10) was orally treated for five days with 100 mg/kg of curcumin diluted in vegetable oil, and the other group (n=10) was treated with vegetable oil (control). Statistical difference was observed in the levels of creatinine, hematocrit, hemoglobin, and red blood cells count between the control and curcumin treated group. However, despite these differences, the values observed were within the normal ranges for of Wistar male rats. Histopathological alterations were not observed. Therefore, our results show that curcumin is relatively non-toxic and, because of its demonstrated pharmacological effects, it can be developed as a promising candidate drug.

Key words: Curcumin, toxicity, side effects.

INTRODUCTION

Curcumin is a phytochemical of a yellow-orange color obtained from the root of the *Curcuma (Curcuma longa)*, herbaceous rhizomatous plant of the ginger family (Zingiberaceae) (Lal, 2012). This polyphenol compound

is widely used as an anti-inflammatory (Kawamori et al., 1999; Satoskar et al., 1986; Srimal and Dhawan, 1973), antitumor (Brouet and Ohshima, 1995; Kunnumakkara et al., 2007), and antioxidant (Asouri et al., 2013). The

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compound demonstrates hepatoprotective and hypoglycemic properties (Goel et al., 2008). Several studies have confirmed its nephron-protecting activity in concomitant use (200 mg/kg orally) with notoriously nephrotoxic agents such as gentamicin (Farombi and Ekor, 2006), acetaminophen (200 mg/kg intraperitoneal curcumin (Cekmen et al., 2009), and vancomycin (200 mg/kg of curcumin twice a day orally) (Ahmida, 2012).

It is believed that curcumin exerts its protective activity by inhibiting the release of interleukins and by activation of macrophages caused by renal ischemia. In addition, curcumin acts as an anti-apoptotic agent by inhibiting the transformation of beta growth factor (TGF- β), a compound which acts as caspase-3 inducer mediating apoptosis in the kidney and liver and leading to cell death (Awad and El-Sharif, 2011). Curcumin (100 mg/kg) was beneficial in acute and chronic poisoning with carbon tetrachloride (Park et al., 2000) by decreasing concentrations of alanine transferase and alkaline phosphatase, which are biochemical markers of liver damage (Park et al., 2000). Curcumin was helpful in decreasing alanine aminotransferase in rats with iron-induced hepatotoxicity (Reddy and Lokesh, 1996). Its concomitant use with ethanol in rats prevented liver alterations caused by ethanol (Bao et al., 2010). In this situation, curcumin acts as a powerful antioxidant, increasing the concentration of glutathione through the increased activity of glutathione peroxidase and glutathione-S-transferase (Rong et al., 2012).

Curcumin is also an excellent therapeutic option in association with cytotoxic anti-tumor agents. It showed antioxidant capacity by inhibiting apoptosis caused by doxyrubicin in cardiac cells (Hosseinzadeh et al., 2011) and optimized antitumor activity of doxorubicin by removing the main route of caspases activation (Sadzuka et al., 2012). Curcumin crosses the blood-brain barrier and is considered as a neuro-protective agent against ischemic damage and neurodegenerative diseases (Guangwei et al., 2010).

Despite its pharmacological potential, data on the safety and rational use of curcumin are still scarce. An ethanolic extract of curcumin demonstrated hepatotoxicity in rats, when administered at concentrations between 0.2 and 1% (Pintão and Silva, 2008) and iron sequestering activity leading to decreased hematological parameters in rats (Jiao et al., 2009). Because curcumin is widely used, especially in conjunction with toxic drugs, the verification of its toxicity is relevant. This study evaluated the effects of curcumin toxicity through hematological and biochemical parameters and histopathology of hepatic and renal tissues in male rats.

MATERIALS AND METHODS

Reagents

The following reagents were used in the study: curcumin (Sigma®);

corn vegetable oil; Labtest® commercial urea EC kit, Ref. 27; Labtest® commercial creatinine K kit, Ref. 35; Labtest® commercial ALT/GPT Liquiform kit, REF. 108; Labtest® commercial alkaline phosphatase Liquiform kit; Ref. 79. Hemoglobin Labtest Reference © Ref. 47.

Animals

Wistar adult male rats (*Rattus norvegicus*) were used in the experiments. All animals weighed approximately 300 g, were housed in 3 per cage in a temperature controlled room, and received food and water *ad libitum*. The animals were acclimated for 30 days before the start of the study. The experiment was approved by the Animal Ethics Committee of the Midwestern State University under opinion/030 2012 and in compliance with regulations to ensure minimum animal stress. The animals were divided into two groups (n = 10): the experimental group was treated with curcumin (100 mg/kg/VO) diluted in corn oil for 5 days, and the control group was treated with corn vegetable oil for 5 days (5 ml/kg of live weight).

Hematological and biochemical tests

At the end of the experiment, animals were euthanized in a carbon dioxide chamber and decapitated. Blood samples were collected for blood count and biochemical measurements, and kidney and liver tissues were harvested for histopathological examination. Hematological alterations were evaluated through hematocrit, total red blood cells count were counted in Neubauer chamber (Gomes et al., 2007). Plasm protein was estimated using manual refractometer (Gomes et al., 2007), hemoglobin was valued by cyanmethaemoglobin method. The reading was performed using a spectrophotometer at wavelength of 540 nm. Leukocytes were counted in Neubauer Chamber (Thrall, 2007). The biochemical tests of serum were performed using a semi-automatic biochemical analyzer (BIOPLUS 200®) and commercial Labtest® kits. Alanine aminotransferase activity was verified according to standard methodology using pyridoxal phosphate (Reitman and Frankel, 1957). Urea was measured by the enzymatic method (Searcy et al., 1961). Creatinine was measured by the kinetic measurement of Jeffé (Myers et al., 2006).

Histological analysis

Tissue samples from kidney and liver were collected and stored in 10% buffered formalin solution. Tissues were sectioned, and stained with hematoxylin and eosin (HE). Two histological sections were obtained from each kidney sample, and one slide with three histological cuts was obtained from liver samples. Samples were evaluated by optical microscopy (Alves, 2002). Lesions were observed qualitatively by checking the presence of morphological alterations in cells and inflammatory infiltrates.

Statistical treatment

The quantitative variables were described as mean and standard deviation. Statistical tests were used in the comparison of independent variables and comparison between curcumin and vehicle in the hematological toxicity analysis. The significance level of 5% was adopted, and the Student's test was used. The analyses were carried out in the Statistical Package for the Social Sciences (SPSS) software version 20.0 (IBM, Armonk, New York, United States).

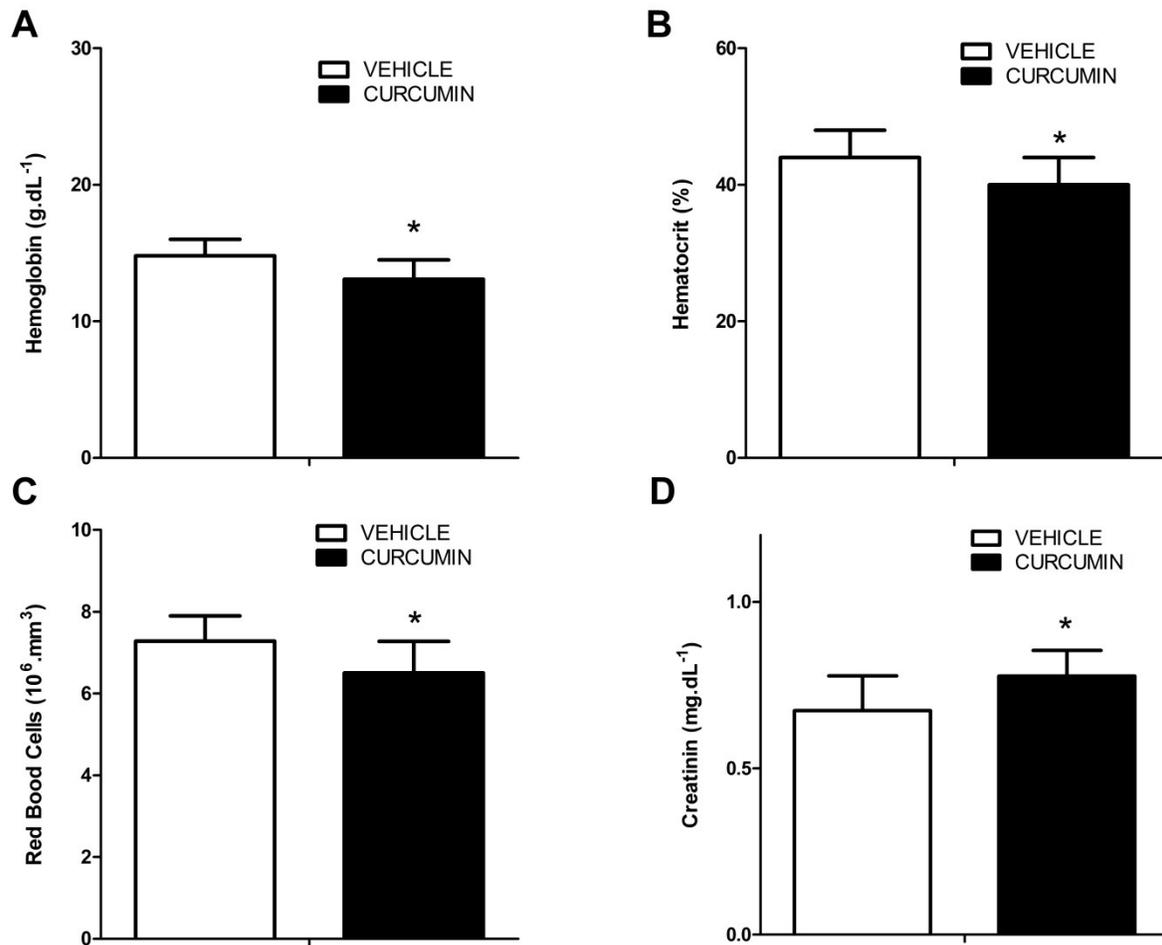


Figure 1. Hemoglobin (A), hematocrito (B), red blood cells count (C) and creatinine (D) in Wistar rats orally treated with 100 mg/kg curcumin for five days and in control animals (n = 10) p < 0.05 in Student's statistical test.

RESULTS AND DISCUSSION

This study evaluated the effects of curcumin toxicity through hematological and biochemical parameters and histopathology of hepatic and renal tissues in male rats. Hematological toxicity was evaluated by the presence of anemia; the analysis showed statistical difference in hematocrit, hemoglobin, and red blood cell between the groups (Figure 1). The decrease in hematological parameters indicates anemia, a condition which can result from toxicity. Mahmoud and Elbessoumy (2013) administered curcumin in rats (600 mg/kg for 6 weeks) and observed hematological alterations, however, no statistical significance was observed when compared with the control group: their observed values were within normal range. The use of curcumin in rats at 0, 0.2, 0.5 and 2.0%, this last concentration corresponding to the highest dose used in clinical studies in humans (8 to 12 g per day), did not lead to alterations in hematological parameters in animals supplemented with iron. However, animals treated with an iron deficient diet presented

hypochromasia of red blood cells and microcytosis, signs of anemia, suggesting that curcumin acts on the hematopoietic system in a dose-dependent mode, which may be suggestive of toxicity (Jiao et al., 2009).

Measurements of blood alanine aminotransferase (ALT) and alkaline phosphatase were conducted to test liver function (Figure 2). ALT is present in hepatocytes and is released upon injury or destruction of these liver cells (Motta, 2000). It is considered a highly specific hepatic enzyme and an increase in concentration of this enzyme is usually proportional to the number of injured cells, suggesting membrane injury due to necrosis in hepatocytes (Thrall, 2007). Alkaline phosphatase is present in bile canaliculi, renal tubules, spleen, bones, and placenta; the predominant form in blood serum is that from liver and bones. It is elevated in cases of biliary tract disorder and cirrhosis (Motta, 2000). In this study, no statistical differences were observed between the levels of ALT and alkaline phosphatase in the experimental and control groups. This result indicates that curcumin does not have hepatotoxic activity and

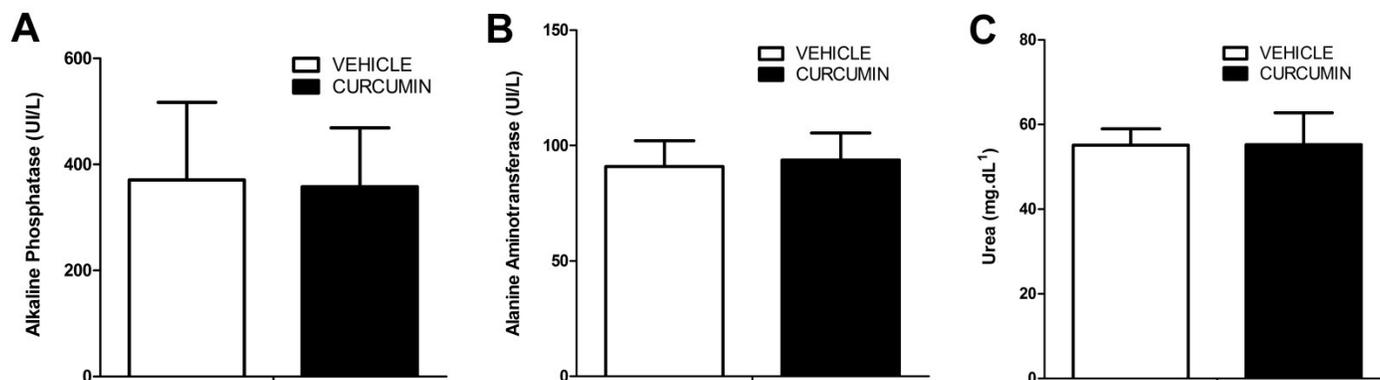


Figure 2. Alkaline phosphatase (A), alanine aminotransferase (ALT) (B) and Urea (C) in Wistar rats orally treated with 100 mg/kg curcumin for five days and in control animals (n = 10) $p < 0.05$ in Student's statistical test.

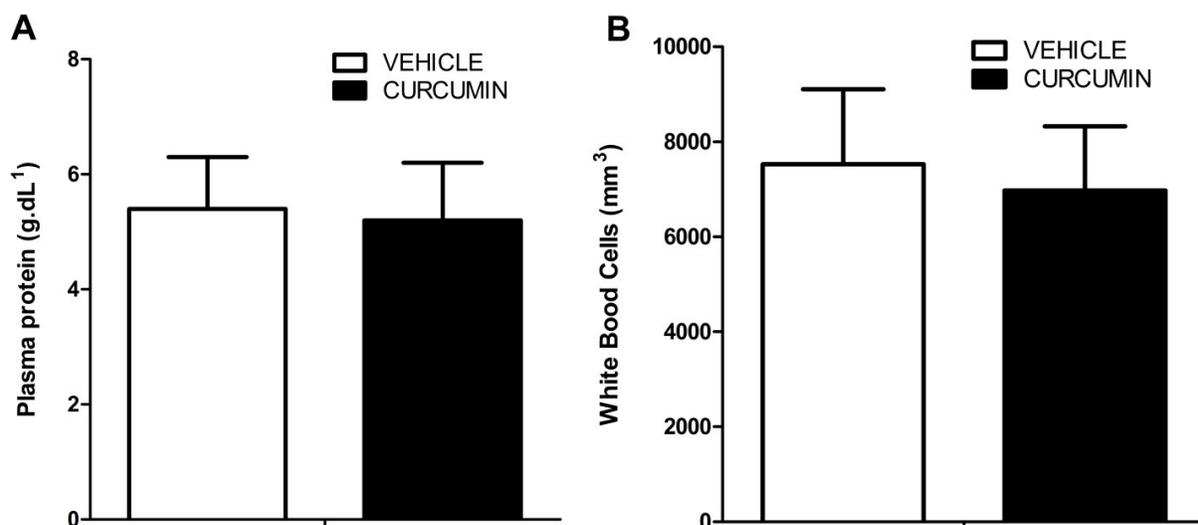


Figure 3. Plasma Protein (A) and White Blood Cells (Leukocytes) (B) in Wistar rats orally treated with 100 mg/kg curcumin for five days and in control animals (n = 10) $p < 0.05$ in Student's statistical test.

corroborates results from other studies in rats that received curcumin (600 mg/kg/VO/6 weeks) (Mahmoud and Elbessoumy, 2013).

Urea and creatinine were measured as blood metabolites to evaluate renal function. Urea is one of the blood parameters used to assess glomerular filtration because most of the urea in the blood is excreted in the urine through this filtration. Consequently, the decrease in glomerular filtration rate leads to increased levels of blood urea (Thrall, 2007). Plasma creatinine is derived from muscle catabolism excretion of plasma creatinine is exclusively renal, and is not reabsorbed or reused. Decrease in renal flow causes its buildup in the bloodstream indicating renal deficit (González and Scheffer, 2003).

Statistical difference was observed in the measurements of urea and creatinine between the studied groups ($p < 0.05$); however, the creatinine values

remained within the normal values found in the literature, 0.5 to 1.5 mg/dl in both groups (Thrall, 2007) (Figure 3). Therefore, despite the statistical difference, no clinical alterations occurred based on the histological examinations and parameters obtained within the normal range.

Histological alterations were not observed. The histological sections show hepatic tissue with an overall preserved architecture, absence of fibrosis or necrotic-inflammatory activity, preserved porta space, without aggression on the limiting plate, and hepatocytes arranged in regular axes. The renal histological sections did not show alterations: all animals presented renal parenchyma with clusters in adequate number, without histological alterations and patent capillary loops. No alterations were observed in interstitial tubules. The histopathological results confirm the absence of liver and kidney toxicity (Figure 4).

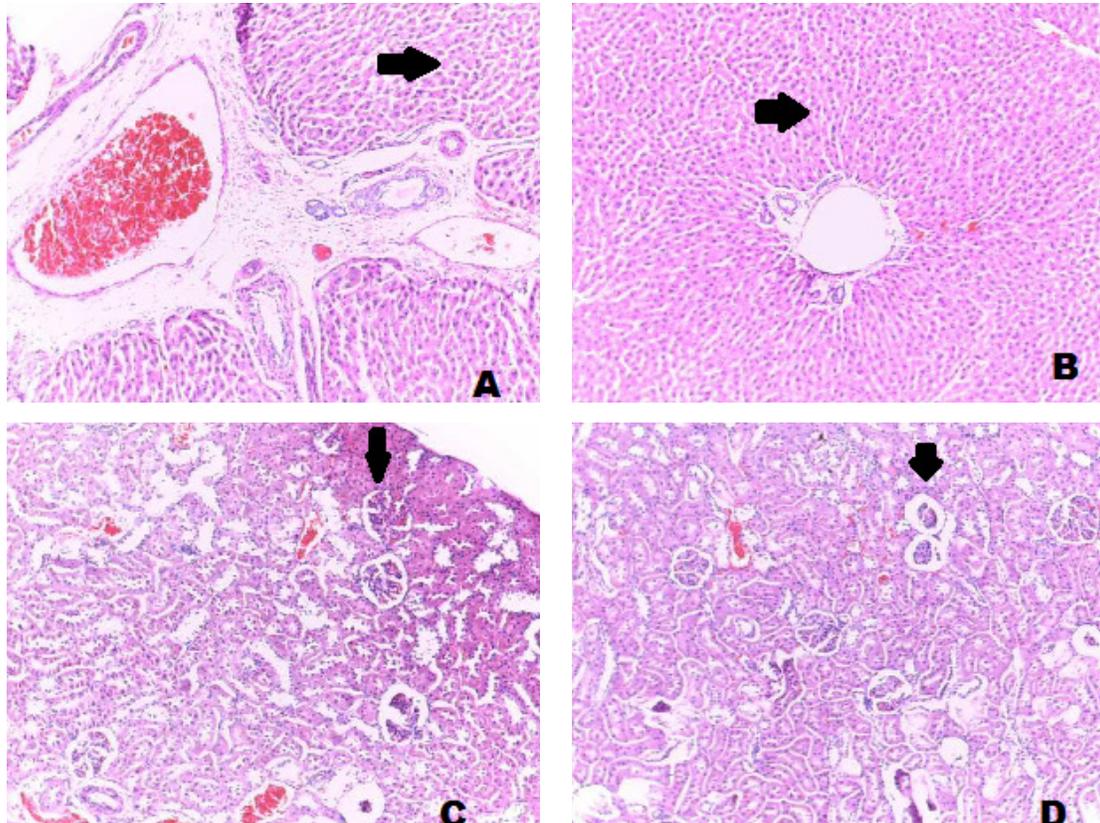


Figure 4. Photomicrography (HE-100×) of a liver section in the control group (A) and experimental group (treated with 100 mg/kg of curcumin) (B); kidney section in the control group (C) and experimental group (D). Normal hepatic parenchyma (Figures A and B – arrows). Kidney without alterations in renal parenchyma and interstitial-tubules (Figures C and D - arrows).

Conclusions

The curcumin regimen evaluated in this study did not cause hepatic damage. The animals treated with curcumin showed statistically different hematological parameters and creatinine levels when compared to those in the control group but values remained within reference values for the the Wistar specie; no difference in urea levels or clinical alterations were observed in these animals suggesting no curcumin toxicity. Potential chronic effects from the use of curcumin could not be evaluated because of the short treatment period used in this study. Thus, this study presents information that support the evaluation of curcumin as a promising candidate drug for several therapeutic applications because it shows low toxicity.

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Conflict of Interest

The authors have not declared any conflict of interest.

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