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Original Research Article

Effect of curcumin and cacao extract on interleukin-6 expression on ribociclib-induced hepatotoxicity in rats

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Abstract

Purpose: *To assess the relationship between interleukin-6 (IL-6) expression and the protective effect of curcumin and cacao extract on hepatotoxicity caused by ribociclib.*
Method: *Sixty adult male albino rats (9 – 10 weeks old) were utilized in this experiment. The rats were separated into six groups (n = 10 each). Group 1 (control) rats received 2 mL of normal saline daily. Group 2 rats were administered 5 mg/kg ribociclib and group 3 rats received 5 mg/kg ribociclib with a cacao dose of 200 mg/kg daily. Group 4 rats were administered 5 mg/kg ribociclib treated with curcumin (200 mg/kg) daily. Groups 5 and 6 were administered cacao and curcumin doses of 200 mg/kg daily. All rats were sacrificed and liver immunohistochemical and histological examinations were performed.*
Result: *The findings demonstrated that ribociclib administration caused liver damage and morphological changes at a histological level in liver tissue. The immunohistochemical investigation revealed that IL-6 levels were significantly increased in group 2 and control group ($p < 0.05$). Furthermore, co-administration of curcumin and cacao with ribociclib suppressed the levels of IL-6 expression in the liver tissue of rats compared to control group 1.*
Conclusion: *Co-administration of curcumin and cacao protects the liver against ribociclib-induced liver damage. It is necessary to determine the effect of long-term administration of these extracts on the functions of some organs such as the liver and kidney.*

Keywords: *Ribociclib, Hepatotoxicity, Curcumin, Cacao, Interleukin 6 (IL-6)*

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INTRODUCTION

In biological systems, reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as superoxide, hydroxyl, and nitric oxide radicals, damage DNA and lead to the oxidation of lipids and proteins in cells [1]. Normally, the human antioxidant system scavenges these radicals, maintaining the balance between oxidation and anti-oxidation. However, exposure to cigarette smoking, alcohol, radiation, or chemotherapeutic drugs induces the production of excessive ROS and RNS, which disrupt the balance between oxidation and anti-oxidation and lead to chronic and degenerative diseases [1].

Consuming antioxidants could ameliorate the damage caused by oxidative stress by inhibiting the initiation or propagation of oxidative chain reaction, acting as free radical scavengers, quenchers of singlet oxygen, and reducing agents [1]. Plant compounds are associated with biological and therapeutic properties, including antioxidant, anticancer, anti-inflammatory, and antibacterial activities [2]. Several phenolic compounds, such as flavonoids, curcumins, and tannins, have been shown to function in inflammatory pathways by inhibiting proinflammatory enzymes like cyclo-oxygenases and lipoxygenases or scavenging free radicals [2].

The imbalance of reactive oxygen species (ROS) leads to several pathologies, including hypertriglyceridemia, cancer, neurodegenerative diseases, diabetes, skin illnesses, aging, wound healing, and cardiovascular diseases. Antioxidant enzymes and natural dietary antioxidants minimize negative effect on health due to the imbalance in ROS and oxidative stress [3]. Curcumin, a natural phenolic molecule, is beneficial for treating chronic conditions because of its anti-inflammatory characteristics [5]. In addition, curcumin, a diarylheptanoids compound, is a natural active polyphenol extracted from Zingiberaceae, including *Curcuma radix, Curcuma longa* rhizoma, *Curcuma rhizoma,* and *Tacori tatarinowii* rhizoma [4]. Curcumin has sufficient and reliable safety with non-toxic properties. Furthermore, it is one of the most widely used natural edible colorings in the world and is authorized by the United States Food and Drug Administration (FDA) for use as a food additive in many countries [5].

Cocoa (*Theobroma cacao* L.) is a rich source of biologically active compounds, such as polyphenols, which are known to act as natural antioxidants. Phenolic compounds are one of the most important groups of secondary metabolites directly related to the sensory characteristic colour and bitter taste and many beneficial health effects of raw cocoa beans and their derived products [6,7].

A previous study showed that ribociclib, a cyclin-dependent kinases 4 and 6 (CDK4/6) inhibitor, had powerful antineoplastic effects in hepatocellular carcinoma with an elevated level of retinoblastoma expression [8], showing that retinoblastoma protein may be employed as a clinical response measure when medicines like ribociclib are used to treat hepatocellular cancer. CDK4 and CDK6-targeting drugs might potentially provide a medicinal resource to cure hepatocellular cancer [9]. Interleukin-6 (IL-6), a cytokine, has been linked to several types of cancer, with higher levels found in patients with liver, breast, and multiple myeloma. IL-6, produced by various cells, plays a role in hepatocellular carcinoma (HCC), the most prevalent liver tumor. The purpose of this research was to investigate the connection between IL-6 expression and the protective effect of curcumin and cacao extract on hepatotoxicity caused by ribociclib.

EXPERIMENTAL

Preparation of antioxidants

Curcumin from powdered *Curcuma longa* and antioxidants from a mixture of turmeric and cocoa extract was purchased from Sigma, Germany. Materials containing antioxidants were macerated for three days in 80 % methanol [10], filtered, and slightly concentrated using a rotary evaporator to extract the methanol. Following freeze-drying to produce a crude extract, the little volume was kept at degrees Celsius until needed. The rats were given 200 mg/kg daily of natural extracts of cocoa or curcumin orally [11]. As previously reported, a 0.1 % carboxymethylcellulose (CMC) solution included uniformly distributed ribociclib powder [12-15].

Animal model

Sixty adult male albino rats (9 – 10 weeks old) were obtained from the animal house at Jordan University of Science and Technology in Irbid, Jordan. Animals were separated into six groups of 10 rats each and allowed to acclimate in a room with adequate ventilation, on a 12-h light/dark cycle, with unlimited access to food and water. The Zarqa University Institutional Research Board approved this study (approval no. 8023) which was conducted by following the guide for the care and use of laboratory animals [15,16].

Animals were divided into the following groups:

Group 1: (n = 10) rats with free access to rat pellets and orally administered saline as a placebo were considered the normal controls.

Group 2: rats administrating 5 mg/kg ribociclib (n = 10).

Group 3: rats administrating 5 mg/kg ribociclib (n = 10) treated with a 200 mg/kg daily dose of curcumin.

Group 4: rats administrating 5 mg/kg ribociclib treated with a 200 mg/kg daily dose of cacao uptake extract.

Group 5: curcumin was given to rats orally at 200 mg/kg daily.

Group 6: cacao was administered to rats via oral gavage at 200 mg/kg daily.

In groups 3 and 4, rats were given a single dose of ribociclib via oral gavage. A daily dosage of curcumin (200 mg/kg group 3) and cacao (200 mg/kg group 4) were administered by oral gavage to rats in groups 3 and 4. The rats were sacrificed after the experiment. Their livers were removed immediately. In all the animals, the liver was blocked for histological examination's rats were sacrificed after the experimental period.

Immunohistochemistry procedures

Liver tissues were prepared in a tissue processor, cut, and sectioned onto charged slides. Then, slices were deparaffinized at 65 °C in an oven for one hour. Afterward, the slides were submerged using a hydrating procedure from xylene to distilled water. The sections were exposed to a solution of 1 % hydrogen peroxide in 100 % methanol for 20 min to reduce or prevent the action of naturally occurring peroxidase. To reduce non-specific binding, the slides were rinsed in phosphate-buffered saline (PBS, pH 7.2 – 7.4) for five minutes before being treated with 1 % bovine serum albumin (BSA) for 30 min.

The key antibody and accompanying immunohistochemistry reagents were allowed to reach 37 °C before use. The monoclonal antibody (IL-6, Sakura Company) was diluted 1:100 and then applied on the slides, followed by an hour of incubation in a humid chamber. The slides were washed for five minutes in PBS (pH 7.2 – 7.4) and treated with secondary biotinylated antibodies for 20 min [13].

After washing for five minutes in PBS (pH 7.2 – 7.4), slides were incubated with streptavidin and horseradish peroxidase for 20 min and washed again with phosphate buffer saline (pH 7.2 - 7.4) for an additional five minutes. To evaluate immunohistochemistry reactions, slides were washed via tap water to stop the reaction after being incubated with DAB (3, 3'-diaminobenzidine) until a reaction occurred and a brown residue was produced. Slides were then mounted on a microscope stage after being rinsed with water, dried, and counterstained for 30 sec with hematoxylin [14].

Interpretation of the results

IL-6 expression was assessed by analyzing the pixel intensity from tissue slides using Adobe Photoshop Version 22.3.1. The pixels showed the biomarker as brown and the rest of the tissue as blue. The IL-6 expression ratio was calculated by dividing the number of brown pixels, indicative of the biomarker, by the total number of pixels (brown + blue) [14].

Statistical analysis

Statistical Package for the Social Sciences (IBM, V 22.0) was used for statistical analyses. Data are reported as the mean ± standard error of the mean (SEM). Statistically significant differences were set at p-value ≤ 0.05.

RESULTS

Immunohistochemical expressions of IL-6

Interleukin-6 expression was significantly greater in group 2 treated with ribociclib than in control group ($p < 0.01$). Moreover, IL-6 expression was significantly higher in group 3 (ribociclib + curcumin) than in control group ($p < 0.017$). Between (ribociclib + cacao) group 4 and control group, there was a significant difference ($p < 0.007$). Groups 5 and 6, which consumed cacao and curcumin, had higher IL-6 levels than the controls; however, these differences were not statistically significant ($p > 0.05$; Table 1).

DISCUSSION

Interleukin-6 is generated transiently and rapidly in response to tissue damage and infections and helps the host defend itself by promoting acute phase responses, hematopoiesis, and immunological reactions. It is involved in several physiological functions, including the immune response to infections and the anti-inflammatory response to control of inflammation.

Table 1: The expression levels of IL-6 among study groups

Group	Interleukin-6	*P*-value
Control	0.0078±0.0299	
Ribocilib	0.0152±0.019	0.001*
Ribocilib+curcumin	0.0121±0.027	0.017*
Ribocilib+cacao	0.0122±0.034	0.007*
Curcumin	0.0101±0.043	1.000**
Cacao	0.0104±0.0156	0.924**

Note: *$P < 0.05$, vs control group; **$p > 0.05$, vs control group

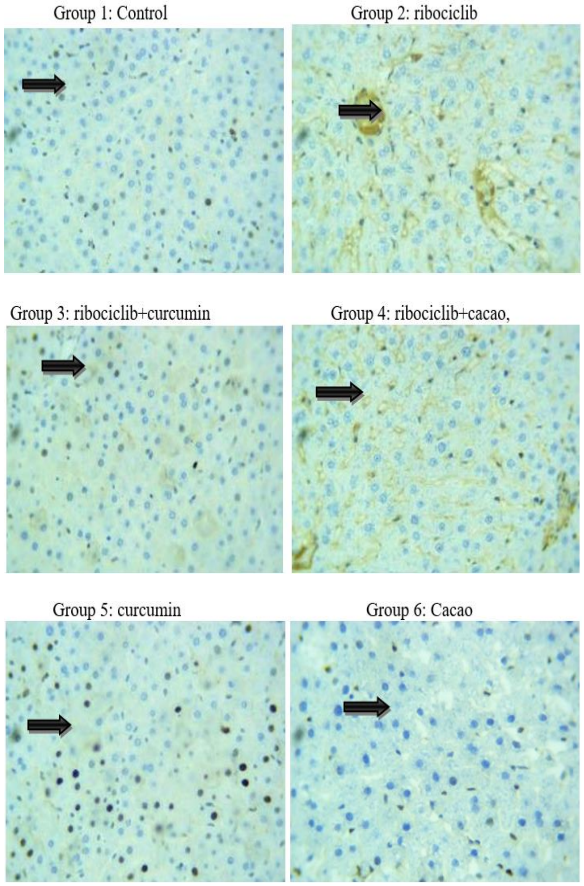

Figure 1: Expression of IL-6 in rat liver tissues from different study groups. Key: Immunohistochemical staining; magnification × 40. The arrows point to the expression of IL-6 in rat liver tissues

Many diseases, including cancer, have been related to abnormal levels of IL-6. Researchers are exploring the link between IL-6 concentrations and liver cancer, using elevated levels as a biomarker and targeting IL-6 signaling pathways as a potential therapeutic approach [15]. Numerous research has focused on bioactive chemicals derived from natural products because of their wide range of therapeutic benefits and low risk of adverse effects. Phenolics, in particular, have the ability to impede the different stages of cancer growth and progression. It follows that their potential use as adjuvant and/or chemotherapeutic drugs is not unexpected [17].

Medicinal plants have long been used to treat liver diseases and serve as effective therapies. However, more scientific evidence is needed to support their benefits. Researchers worldwide have studied the preventive effects of plant extracts against experimentally induced hepatotoxicity [18]. The overproduction of ROS can result in lipid peroxidation and oxidative damage, a recognized side effect of anticancer drugs like ribociclib. These are essential elements for chemically caused liver damage. Exposure to anticancer medications can cause severe liver necrosis and steatosis, making them a common hepatic toxin in animals to explore the hepatoprotective properties of numerous medicinal plants and natural chemicals [18].

The study indicated that the expression of IL-6 was considerably higher in the ribociclib group 2, ribociclib + cacao group 3, and ribociclib + curcumin group 4 compared to control group 1 ($p < 0.05$). The decrease in IL-6 expression in this study suggests and verifies curcumin and cacao extracts' hepatoprotective activity when combined with ribociclib.

Curcumin has antioxidant properties, and oxidative stress contributes to inflammation. By neutralizing ROS and reducing oxidative stress, curcumin may indirectly contribute to the downregulation of IL-6 [14]. Curcumin and cacao components, like epicatechin, have been found to have gastro-protective effects, boosting mucin production and reducing stomach ulcers while downregulating pro-inflammatory cytokines [14,19].

According to recent in vitro studies, cocoa phenolic components have been shown to exhibit a variety of potential anti-inflammatory effects in liver cells, perhaps supporting the cells' chemopreventive efforts against cancer. Furthermore, such processes have yet to be examined in hepatocellular carcinoma (HCC) in vivo research. Studies have demonstrated that cacao and curcumin protect against lead, mercury, cadmium, and copper, among other heavy metals. They also reduce hepatotoxicity and prevent mitochondrial dysfunction [14,19].

Limitations of this study

Discovering anti-cancer drugs from natural sources presents various challenges, slowing the development of effective treatments. The complicated molecular compositions of these natural compounds present considerable problems due to their complexity, making separation, identification, and synthesis challenging and resource-intensive. Furthermore, the efficiency of natural chemicals as anti-cancer medications could be improved by better absorption and distribution in the body, raising worries about their bioavailability. Toxicity is important since some natural compounds can pose dangers, and extensive toxicological testing is needed to ensure patient safety. Furthermore, the one-month duration for this study was not sufficient, the results would have been more

robust if the study had been conducted for more than three months.

CONCLUSION

This study has reported the hepatoprotective effect of curcumin and cacao extracts administered orally to rats with ribociclib-induced HCC. The impact has been achieved by regulating inflammatory cytokines, such as IL-6 and reducing oxidative stress. Future studies to determine the effect of long-term administration of these extracts on the functions of special organs such as the liver and kidney will be required.

DECLARATIONS

Acknowledgement

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None provided.

Ethical approval

The Zarqa University Institutional Research Board approved this study (8023).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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