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" *Review on the Effect of curcumin on methotrexate induced hematological changes in rats*"

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ABSTRACT:

Methotrexate (MTX), once known as amethopterin, is a medication that suppresses immunity and acts as a chemotherapeutic agent. It is used to treat a variety of conditions, including ectopic pregnancies, several types of cancer such as leukemia, lung cancer, breast cancer, osteosarcoma, lymphoma, and gestational trophoblastic disease. The mechanism of MTX action involves the production of reactive oxygen species (ROS), which can suppress bone marrow function, leading to an increased risk of infection and bleeding. Pancytopenia, a condition characterized by blood abnormalities, is the most common side effect.

Curcumin, a powerful antioxidant herb, has multiple beneficial properties, including antibacterial, anti-inflammatory, hypoglycemic, antioxidant, wound-healing, and antimicrobial activities. This study was designed to investigate the hematological effects of MTX and the impact of curcumin on the hematological changes induced by MTX in four groups of rats. The first group served as the control, the second group received curcumin, the third group received methotrexate, and the fourth group received both curcumin and methotrexate for one month. At the end of the study, bleeding time was measured, and blood samples from all rats were examined for complete blood count (CBC), platelet aggregation, superoxide dismutase (SOD) levels, and malondialdehyde (MDA) levels.

Introduction

The WHO defines methotrexate (MTX) as an "essential drug," and it has become widely used as an initial therapeutic option for immunoactive diseases and disorders that cause tissue inflammation. It is a medication that effectively controls inflammation for millions of people worldwide and is also used as a chemotherapeutic agent.1

Methotrexate is a 4-amino, 10-methyl derivative of folic acid in its polyglutamated form. It is a potent drug that inhibits dihydrofolatereductase, an enzyme crucial for reducing dihydrofolate to tetrahydrofolate. In cells, tetrahydrofolate is essential for the synthesis of purines and pyrimidines, and it also contributes to the one-carbon donors required for DNA methylation. Tetrahydrofolate plays a vital role in nucleotide synthesis for DNA and RNA.2 MTX-polyglutamate inhibits the de novo synthesis of purines and thymidylates, thereby disrupting DNA and RNA synthesis.3

MTX significantly affects the hematological system. The drug poses a considerable risk, especially because it targets high-turnover tissues like the bone marrow, causing a halt in synthesis during the S-phase of the cell cycle.4This ultimately leads to cell death, which results in a decline in blood cell production.5

The hypothesis behind methotrexate (MTX) toxicity involves the inhibition of NADPH-dependent mitochondrial enzymes. NADPH is primarily produced by mitochondrial enzymes, and MTX reduces NADPH levels, which are essential for the activity of glutathione reductase. Glutathione reductase plays a crucial role in protecting against reactive oxygen species (ROS) by maintaining reduced glutathione levels. By decreasing both NADPH and glutathione levels, MTX makes hepatocytes more susceptible to ROS, leading to damage in various body tissues.6

MTX is primarily metabolized in the liver and excreted through the kidneys. The primary metabolite of MTX is 7-hydroxy-methotrexate (7-OH MTX). Within 24 hours of ingestion, 7-OH MTX is eliminated through tubular reabsorption and filtration. Bile also excretes distinct amounts of 7-OH MTX. 7This metabolite has a short plasma half-life, resulting in diminished therapeutic efficacy. However, MTX accumulates in healthy tissues, particularly the liver, kidneys, and intestines, leading to severe toxicities, such as kidney damage, liver toxicity, and ulcerative colitis. 8 The toxicity of MTX is dose-dependent and can affect several organ systems, including the kidneys, liver, mucosal tissue, and bone marrow.9

Hematological side effects are among the most serious adverse effects of MTX, as the drug can cause oxidative damage even at low doses. The polyunsaturated fatty acids present in blood cell membranes make them particularly vulnerable to oxidative damage.10

The National Coronial Information System (NCIS) has identified methotrexate (MTX) as a cause of mortality due to its effects on bone marrow suppression. Additionally, hematopoietic cytotoxicity associated with MTX causes up to 25% of patients to abandon therapy, thus increasing the risk of mortality.11

Pancytopenia (including thrombocytopenia, leukopenia, neutropenia, and megaloblastic anemia) is one of the most challenging side effects of MTX. MTX can elevate the possibility of pancytopenia, which may appear spontaneously during therapy, contributing to therapy-related mortality.12

Excess unbound extracellular MTX induces thrombocytopenia by suppressing bone marrow and accelerating platelet death through mitochondrial dysfunction. Thrombocytopenia can result in bleeding from the gums, urinary tract, vagina, and rectum.13

The etiopathogenesis of secondary thrombocytosis induced by methotrexate has been proposed to involve mechanisms such as enhanced splenic mobilization, enlarged megakaryocytes, increased endogenous interleukin-6 (IL-6), other cytokines or catecholamines, and prolonged platelet lifespan.14

Nauman ismat butt et al hypothesized that methotrexate causes thrombocytopenia as a direct consequence. This thrombocytopenia stimulates release of thrombopoietin which increases platelet production as a secondary response.15

Leukopenia can occur within one to three weeks of initiating methotrexate (MTX) treatment. The immunosuppressive effects of MTX increase the risk of developing infections and also enhance the severity of infectious diseases after starting MTX therapy.16

Due to concerns over medication resistance and the side effects of synthetic drugs, there has been growing interest globally in herbal extracts, metabolites, and medicinal cannabis. One such herb is Curcumin (CUR).17 CUR, also known as diferuloylmethane (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione), is widely recognized for its numerous biological properties.18

Curcumin is derived from the rhizomes of *Curcuma longa* (C. longa), a perennial herbaceous plant with yellow blooms, belonging to the Zingiberaceae family. The most common curcuminoids extracted from C. longa are curcumin (diferuloylmethane), demethoxycurcumin, and bisdemethoxycurcumin. Curcumin (CUR) is the primary bioactive compound responsible for turmeric's medicinal effects.19

Dried *C. longa* typically contains carbohydrates (69.43%), proteins (6.3%), lipids (5.1%), minerals (3.5%), and diarylheptanoids (curcuminoids). Curcumin, which imparts turmeric's yellow color, makes up about 2% to 5% of the plant. 20This plant is native to temperate and subtropical regions of Asia, including India, China, Indonesia, Jamaica, Peru, and Pakistan. Curcumin is a bioactive, hydrophobic polyphenolic compound found in turmeric. Historically, curcumin has been used to manage various disorders and is widely used as a spice and natural coloring agent in culinary products.21

Curcumin is available in various forms, including tablets, pills, lotions, energy beverages, detergents, and moisturizers. Numerous clinical studies have demonstrated high tolerance and safety for curcumin or curcuminoids, with doses ranging from

4000 to 8000 mg. Consequently, curcuminoids are generally considered "mostly appropriate" by the United States Food and Drug Administration (FDA).22,23,24

Curcumin has been widely used for treating cardiovascular diseases due to its anti-apoptotic, anti-angiogenic, anti-thrombotic, and antihypertensive properties. 25Studies show that curcumin (50 and 100 mg/kg/day) and tetrahydrocurcumin (THU) effectively reduce arterial and venous stiffness, lower blood pressure, and improve arterial and venous reactivity. These improvements are linked to increased presence of the eNOS protein, as well as elevated nitrate/nitrite levels in the bloodstream and aortic tissue. 26CUR and THU have also been shown to reduce oxidative stress, lower the formation of vascular superoxide, and increase circulating GSH levels and the GSH redox ratio in L-NAME-induced hypertensive rodents.27

Curcumin exhibits neuroprotective properties in both humans and experimental animals suffering from depression and Alzheimer's disease. 28,29 .It has been shown in multiple studies to possess anti-dementia effects.30,31

Furthermore, curcumin has immunomodulatory effects, making it useful in treating rheumatoid arthritis. Its anti-arthritic properties are enhanced by its ability to reduce prostanoid production and suppress the expression of key cytokines like TNF-α and IL-6, which are involved in arthritis. Curcumin also exhibits anti-inflammatory effects by inhibiting the production of pro-inflammatory prostaglandins and leukotrienes, as well as the conversion of linoleic acid to arachidonic acid. It also inhibits the activation of nuclear factor kappa B (NFκB), leading to reduced cyclooxygenase-2 (COX-2) synthesis.32

Because curcumin helps manage diabetes and has an anti-hyperlipidemic effect, it is considered an effective treatment for metabolic syndrome.33Additionally, curcumin possesses anti-lithogenic properties and can reduce heavy metal absorption.34

Curcumin (CUR) is a natural ingredient that has been found to possess cancer-preventive properties in both pre-clinical and post-diagnosis studies. CUR's tumor prevention capabilities include inhibiting carcinogenesis, angiogenesis, and tumor growth and metastasis by triggering apoptosis in cancer cells while protecting healthy cells. CUR also mitigates the negative effects of chemotherapy and radiotherapy, improving the quality of life for affected individuals.35

Numerous studies have shown that CUR prolongs the survival duration of patients and lowers tumor marker levels. It exhibits a broad range of pharmacological activities against chronic diseases by suppressing cell proliferation and metastasis. These effects are mediated through the downregulation of several factors, including NF-κB, IL-1β, TNF-α, hypoxia-inducible factor (HIF-1α), and other stimulated factors.36,37,38

The cell membranes of blood cells are particularly vulnerable to oxidative stress due to their high polyunsaturated fatty acid content. CUR's protective mechanism primarily involves its ability to reduce reactive oxygen species (ROS) levels, thereby shielding blood cells from damage caused by MTX (methotrexate).

However, CUR's specific impact on reducing hematological cytotoxicity is challenging to determine, as it interacts with various factors regulating transcription, enzymatic activities, second messenger systems, and signaling pathways. Nonetheless, CUR is believed to exert its protective effects by promoting erythropoiesis.39

CUR is a potent NF-κB inhibitor that promotes TNF gene transcription, thereby facilitating erythropoiesis. It also stabilizes lysosomal membranes, which enhances the viability of blood cell membranes. The restoration of platelet count, total white blood cell (WBC) count, and differential counts suggests that CUR may mitigate hematological abnormalities caused by MTX, particularly in conditions such as rheumatoid arthritis. One possible mechanism for this is CUR's ability to inhibit the production of leukotrienes and interleukins, which play a role in inflammation. These findings suggest that lowering the dosage of MTX and adding CUR may reduce the likelihood of hematological damage.40

Furthermore, CUR may inhibit arachidonic acid production, reduce thromboxane synthesis, and modulate neutrophil and lymphocyte activity. It has been shown to effectively quench ROS, protecting blood cells from oxidative damage.41

Studies also indicate that CUR reduces hematocrit and hemoglobin levels in rodents on an iron-deficient diet, suggesting that it could contribute to anemia in individuals with insufficient iron or those suffering from cancer or chronic diseases.42 In addition, in vitro studies have demonstrated that CUR prevents platelet aggregation.43,44

Reactive oxygen species (ROS), also known as free radicals, are unstable molecules that are produced during various bodily processes and are constantly present in the body. Under normal conditions, antioxidant systems eliminate these radicals. However, when these systems are disrupted, radicals can accumulate excessively and contribute to the development of various diseases.45

Free radicals are molecules that contain an unpaired electron in their atomic orbital. Due to this unpaired electron, these radicals are highly reactive and unstable. By donating or receiving an electron, they act as both oxidants and reductants. Free radicals are substances that react with surrounding molecules in an unselective, transient, and highly unstable manner due to their high electrophilicity, which results from the presence of unpaired electrons.46

In 1954, electron paramagnetic resonance spectroscopy was used for the first time to demonstrate that biological systems contain free radicals. Free radicals can be classified into three main categories: reactive oxygen species (ROS), reactive nitrogen species (RNS), and radicals that lack oxygen or nitrogen.47

During processes such as the mitochondrial respiratory chain, phagocytosis, arachidonic acid metabolism, and ovulation and reproduction, highly reactive reactive oxygen species (ROS) are produced. When pathological conditions arise, their generation increases significantly. Numerous pathogenic stimuli have been shown to

release oxygen-free radicals during recovery phases, and these radicals can reach cerebral tissue.48

These reactive species can cause DNA damage and lead to lipid peroxidation. The extremely reactive hydroxyl radical (•OH) can further extract hydrogen atoms from various components, including proteins, lipids, and nucleic acids, resulting in the production of water molecules. Additionally, singlet oxygen (1O2) is a well-researched free radical used in cancer treatment, and it can be generated exogenously by external stimuli such as photosensitization or endogenously by myeloperoxidase (MPO) in the azurophilic granules of neutrophils.49

Through the accumulation of adenosine and the suppression of polyamines, MTX can have anti-inflammatory properties. By producing free radical substances and causing oxidative stress, MTX acts as a caspase activator that targets T lymphocytes. Additionally, MTX is known to affect the function of the redox system.50 Oxidative stress contributes to MTX's toxicity by suppressing antioxidant defenses and generating excessive free radicals.51

MTX impairs the mitochondrial apparatus and increases the production of reactive oxygen species (ROS), which can trigger oxidative destruction of critical intracellular macromolecules, such as proteins and lipids. The observed rise in oxidation of proteins and lipids can be attributed to this process. Furthermore, there is an increase in nitric oxide (NO), a potent free radical that, when produced in excess, can react with and damage proteins, lipids, and DNA. Additionally, NO interacts with superoxide radicals, increasing the production of peroxynitrite, a powerful oxidizing agent that can further exacerbate damage to proteins and lipids. NO also serves as an active mediator that reduces the rate of glomerular filtration and induces renal vasoconstriction.52

MTX also causes the depletion of certain antioxidants, including enzymes such as catalase, arginase, hemeoxygenase 1, glutathione, superoxide dismutase, and glutathione peroxidase. This depletion results in the suppression of the transcription of other antioxidants, such as nuclear factor erythroid 2-related factor 2 (Nrf-2), which compromises the cell's defense mechanisms. Additionally, increased DNA synthesis due to folate deprivation does not compensate for the imbalance in the equilibrium of caspase regulators, BAX and Bcl-2, ultimately promoting apoptosis.53

The human body has evolved an antioxidant defense mechanism that includes metal chelation, free radical scavenging, and enzymatic activities to neutralize reactive species as soon as they are produced, reducing or preventing oxidative damage caused by free radicals. Furthermore, dietary antioxidant intake can sustain sufficient levels of antioxidants in the body.54

Secondary byproducts of regular cellular metabolism include reactive oxygen species (ROS). Oxidative stress is defined as "an unbalanced ratio of oxidizing agents to antioxidants, which leads to harm to molecules by interfering with the redox signaling process and regulation."55

By either enhancing the expression and activity of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), or minimizing the expression or activity of enzymatic processes that generate free radicals, such as xanthine oxidase (XO) and NAD(P)H oxidase, antioxidants can reduce the quantity of reactive species in the cellular structure.56

The two primary modes of action of antioxidants that have been described are as follows: The second process focuses on eliminating the catalysts of reactive species (secondary antioxidants), while the first involves circuit breaking, where primary antioxidants donate an electron to the free radicals, extinguishing a catalyst that initiates the process. Therefore, antioxidants have additional effects in biological systems, employing a variety of mechanisms such as co-antioxidants, metal ion chelation, electron donation, and gene expression modulation.57

There are two types of antioxidants: endogenous and exogenous. Superoxide dismutase and catalase (CAT) are examples of antioxidants that are naturally produced in peroxisomes, along with glutathione and glucose-6-phosphate dehydrogenase. Exogenous antioxidants include selenium, and vitamins such as E, C, A, and curcumin. These exogenous antioxidants support the body by donating electrons, thereby preserving chemical equilibrium. They scavenge reactive species and free radicals. Due to their electron-donating properties, these dietary substances can become saturated after a certain point.58

Curcumins, natural antioxidants, employ various mechanisms to combat free radicals. They can neutralize reactive nitrogen and oxygen species, and influence the activity of enzymes like superoxide dismutase (SOD), catalase, and glutathione (GSH). Curcumin also limits the induction of enzymes that generate reactive oxygen species (ROS), such as cyclooxygenase/lipoxygenase and xanthine oxidase/hydrogenase. Additionally, because curcumin is a lipophilic molecule, it can scavenge peroxyl radicals. As a result, curcumin serves as a chain-breaking antioxidant, much like vitamin E.59

Curcumin (CUR) activates glutathione S-transferase, inhibiting free radical generation and acting as a scavenger of DPPH and ABTS. This helps prevent lipid peroxidation by releasing antioxidants. CUR exhibits antioxidant properties due to its chemical composition.60 Research suggests that CUR is particularly effective in activating macrophages to remove superoxide radicals (nitric oxide and hydrogen peroxide), reduce iron complexation, and prevent lipid peroxidation. CUR's primary mechanisms are utilized in pharmacological and therapeutic applications, making it a promising natural antioxidant for the pharmaceutical and food industries.61

CUR metabolizes through its autoxidation properties, producing deoxygenated metabolites with electrophilic and nucleophilic moieties. The various biological actions of CUR are attributed to its β-dicarbonyl group as a metal chelator, its keto-ene moiety as a Michael acceptor, and its phenolic hydroxyl group as an antioxidant and hydrogen donor.62

Conclusion:-

Curcumin improves the hematological effects induced by methotrexate by enhancing antioxidant activity and reducing oxidative stress markers produced by methotrexate.

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