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Therapeutic Effect of Lebanese Cannabis Oil Extract in the Management of Sodium Orthovanadate-Induced Nephrotoxicity in Rats

By

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Therapeutic Effect of Lebanese Cannabis Oil Extract in the Management of Sodium Orthovanadate-Induced Nephrotoxicity in Rats

Christabel Boutros Habchy

ABSTRACT

The current study investigated the therapeutic effect of Lebanese Cannabis oil extract against Sodium orthovanadate-induced nephrotoxicity. Sodium orthovanadate is a non-selective inhibitor of protein tyrosine phosphatases that are known to play major modulator roles in cell signaling and survival. Kidney is a major target for the toxicity of Sodium orthovanadate causing several types of renal injury, including glomerulosclerosis, inflammation and tubular damage. The effect of Cannabis oil on Sodium orthovanadate-induced nephrotoxicity was studied in vivo using Sprague Dawley male rat model. Rats were intraperitoneally injected with 10mg/Kg Sodium orthovanadate for 10 days followed by 5mg/Kg, 10mg/Kg, or 20mg/Kg intraperitoneal injection of Cannabis oil extract starting day 4 till day 10. Body weight of rats were monitored during the study and clinical parameters including serum urea, creatinine, and electrolytes were measured as well as kidney and heart pathology. Rats that were injected with Sodium orthovanadate displayed a marked reduction in body weight, increase in serum creatinine and urea in comparison to the control group. All doses of Cannabis oil caused significant decrease in serum urea, as well as in serum creatinine at a dose of 20mg/Kg. In addition, a marked reduction in renal vascular dilatation, scattered foci of acute tubular necrosis, and numerous mitosis in tubular cells was observed in Cannabis oil treated rats (20mg/Kg) as compared to the Sodium orthovanadate- treated group. In conclusion, the primary findings demonstrate a potential therapeutic effect of Cannabis oil on kidney damage induced by Sodium orthovanadate.

Keywords: Sodium Orthovanadate, Cannabis Oil Extract, Nephrotoxicity, Renal Biomarkers.

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List of Abbreviations

2-AG	2-arachidonoyl-*sn*-glycerol
AEA	*N*-arachidonoyl ethanolamide
AKI	Acute Kidney Injury
BUN	Blood Urea Nitrogen
C. sativa	Cannabis sativa
CB_1	Cannabinoid subtype 1
CB_2	Cannabinoid subtype 1
CBD	Cannabidiol
CKD	Chronic Kidney Disease
COX	Cyclooxygenase
EC	Endocannabinoid
ECM	Extracellular matrix
ERK	Extracellular Signal Regulated Kinase
GFR	Glomerular Filtration Rate
JNK	c-Jun amino-terminal kinases
KIM-1	Kidney Injury Molecule-1
MAPK	Mitogen- Activated Protein Kinase
Na_3VO_4	Sodium orthovanadate
PGG_2	Prostaglandin Endoperoxide G_2
PGH_2	Prostaglandin H_2
PGI_2	Prostacyclin
PLA_2	Phospholipase A_2 Enzyme
PTP	Protein Tyrosine Phosphatase
THC	Tetrahydrocannabinol

CHAPTER ONE
INTRODUCTION

1.1 Normal Physiology of the Kidneys

The renal system consists of the kidney, ureters, and the urethra. The main role of the kidneys is to filter fluid from renal blood flow, allowing toxins, metabolic waste products, and excess ions to be expelled while maintaining the blood's important components (Costantini & Kopan, 2010). The kidneys are in charge of preserving fluid and solute balance in the face of a variety of environmental factors and dietary changes. By adjusting the blood's concentration of water, solutes, and electrolytes, the kidney controls the plasma osmolarity (Cupples, 2007). Furthermore, the main functions of kidneys include reabsorption of vital nutrients which include glucose, amino acids, and electrolytes, in addition to the secretion of Erythropoietin (regulate production of red blood cells in bone marrow), renin (control blood pressure), and Calcitriol (active form of vitamin D essential for bone maintenance) (Robson, 2014).

1.2 Nephrotoxicity

1.2.1 Introduction to Nephrotoxicity

The kidney is the primary control system of the human body, maintaining homeostasis. It plays an important role in the regulation of the extracellular environment, particularly in the excretion of toxic metabolites and drugs (Ferguson et al., 2008). Kidney disease is a major public health issue that imposes significant socioeconomic burdens on affected individuals and their families. Chronic kidney disease (CKD) is a condition characterized by a gradual loss of kidney function over time. Renal fibrosis and renal failure can occur as a result of progressive renal disease.

Kidney disease is a worldwide public health problem especially in developed countries where more than 10 % of adults have CKD (López-Novoa et al., 2010). An estimated 5 to 10 million patients die from kidney disease each year (Luyckx et al., 2018). Additionally, Lebanon has one of the highest rates of dialysis prevalence in the world,

with an estimated 777 patients per million people compared to 410 patients per million people worldwide (Aoun & Ammar, 2019).

1.2.2 Acute Kidney Injury

Acute kidney injury (AKI) is defined as a sudden and often reversible decrease in kidney function as measured by glomerular filtration rate, increased creatinine, or decreased urine volume (Muroya et al., 2018). AKI pathophysiology has traditionally been divided into three categories: prerenal, renal, and postrenal. Each of these categories is associated with a variety of causes (Moresco et al., 2018). Intrinsic renal causes include conditions that affect the glomerulus or tubule, such as acute tubular necrosis, acute interstitial nephritis, and glomerulonephritis (Goyal et al., 2017).

The pathogenesis of acute kidney injury is etiology-driven. The common endpoint in all types of acute tubular necrosis is a cellular insult caused by ischemia or direct toxins, which results in effacement of the brush border and, eventually, cell death, effectively destroying tubular cell function. The mechanism of injury in glomerulonephritis, on the other hand, may be due to direct immune-mediated injury of the vessels or immune complex deposition, resulting in an immune response and damage to the glomeruli (Crabbs, 2018).

1.2.2.1 Drug-Induced Nephrotoxicity

CKD can be caused by a variety of factors, including comorbidities like diabetes and hypertension, inflammatory glomerular diseases, environmental factors, diet, genetics, and drugs. The kidneys are major target for endogenous and exogenous toxicants. Approximately 20% of nephrotoxicity is caused by toxic chemicals or drugs (Porter, 2003). The incidence of drug-induced nephrotoxicity has been increasing with the increasing number of drugs. Chemical models have been developed to study renal disease. As a result, several mechanisms of drug-induced nephrotoxicity have been described, including inflammation, tubular cell toxicity, changes in glomerular hemodynamics, and crystal nephropathy (Ferguson et al., 2008).

1.2.3 Renal Fibrosis

Renal fibrosis is a common pathological feature in CKD and a major predictor of renal insufficiency (Farris et al., 2011). Development of CKD is evidenced by the loss and replacement of renal cells by extracellular matrix (ECM) in glomeruli and interstitium (Fogo, 2001). Renal fibrosis is caused by an imbalance in the synthesis and degradation of ECM, as well as the accumulation of activated fibroblasts, macrophage and lymphocyte infiltration, and mesangial cell and podocyte apoptosis and survival (Stahl & Felsen, 2001). Glomerulosclerosis is manifested by the accumulation of matrix proteins such as such as collagens I, III, IV, and fibronectin in the glomerulus whereas tubulointerstitial fibrosis is manifested by the presence of matrix proteins replacing the tubules and/or surrounding interstitium (Gewin, 2018).

Renal fibrosis is frequently associated with vascular obliteration, tubular atrophy, and kidney shrinkage, all of which cause changes in the kidney's functional, mechanical, and molecular properties (KDIGO, 2009). Vascular rarefaction reduces renal perfusion and oxygen delivery, resulting in tissue hypoxia. ECM deposition and accumulation, as well as tubular atrophy, limit water molecule mobility and increase kidney stiffness and macromolecule content (Jiang et al., 2019).

1.2.4 Role of Cyclooxygenase in Renal Injury

Cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) are the most studied cyclooxygenase isoforms. COX-2 has been found to play a significant role in renal function, primarily in the medullary region and, to a lesser extent, in the cortical region (Batlouni, 2010). In healthy kidneys, it is expressed in smooth muscle cells of afferent and efferent arterioles, the endothelium, the renal artery, interstitial fibroblasts, and podocytes (Khan et al., 1998). It is also found in the thick ascending limb of the Henle loop and the macula densa, which moderates the interaction between glomerular filtration and proximal reabsorption and regulates sodium and potassium ion levels in the distal tubule lumen via the renin-angiotensin-aldosterone system (Nørregaard et al., 2015).

Cyclooxygenase is the main enzyme that catalyzes prostaglandin synthesis from arachidonic acid. This path consists of three major steps. First, the arachidonic acid in the cell membrane's lipid bilayer is hydrolyzed by the phospholipase A_2 enzyme (PLA_2) in response to physical, chemical, inflammatory, or mitogenic stimuli. Second, COX oxygenates arachidonic acid, producing prostaglandin endoperoxide G_2 (PGG_2). Finally, peroxidase converts PGG_2 into an unstable intermediate, prostaglandin H_2 (PGH_2). PGH_2 is converted to various prostanoids by tissue-specific isomerases, including prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2) (Goetz Moro et al., 2007).

PGE_2 is produced by the tubular epithelium and interstitial cells and is expressed in the renal tubules, where it regulates chloride and sodium transport in the Henle loop, whereas PGI_2 is found in the renal cortex, where it regulates glomerular filtration rate and renin secretion (Hao et al., 2014). In vitro study revealed that concentration of 100 uM vanadium compounds increased COX-2 expression. *In vivo*, that high concentration was shown to interfere with mitochondrial function and disrupt cellular respiration, as well as cause severe acute renal failure and liver damage (Boulassel et al, 2011).

1.2.5 Role of Mitogen-Activated Protein Kinases in Kidney Disease

Many intracellular compartments depend on the intracellular signaling cascade as a primary route of communication between the plasma membrane and regulatory targets. Activation of protein kinases is one major mechanism involved in signal transduction in various cellular processes through the addition of phosphate groups to serine, threonine, and tyrosine amino acid residues *(*Stambe et al., 2003). This process is known as phosphorylation.

Mitogen-activated protein kinases (MAPKs) are a major intracellular signaling cascades which belong to a large group of serine/threonine protein kinases. MAPKs modify the phosphorylation status of various proteins such as transcription factors, kinases, and other enzymes in combination with several signaling pathways influencing gene expression, cell metabolism, cell division and consequently cell survival (Cassidy et al., 2012). One or more MAPKs are activated in the transmission of extracellular signals to intracellular targets. Following the binding of an external

stimuli to the receptor on the cell surface, the associated protein tyrosine kinase in the intracellular domain of the receptor is activated and then signaling events are started.

Activation of MAPK is based on three steps. First MAPK kinase kinase (MAP3K), which are serine/threonine kinases, phosphorylates and activates MAPK kinase, known as MAP2K, which in turn phosphorylates one or more MAPKs through dual phosphorylation on threonine and tyrosine residues (Orton et al., 2005; Rubinfeld & Seger, 2005). Once activated, MAPKs can phosphorylate several different intracellular targets including transcription factors, nuclear pore proteins, membrane transporters, cytoskeletal elements, and other protein kinases on serine or threonine residues followed by a proline (Raman et al., 2007).

The dysregulation of normal MAPK signaling have been involved in various human diseases including inflammation, cardiovascular disease, cancer, and both acute and chronic kidney disease (Cuarental et al., 2019). Till today, five different groups of MAPKs have been identified in mammals: extracellular signal-regulated kinases (ERKs) 1 and 2 (ERK1/2), c-Jun amino-terminal kinases (JNKs) 1, 2, and 3, p38 isoforms α, β, γ, and δ, ERKs 3 and 4, and ERK5 (Chen et al., 2001). ERK 1/2, JNKs, and p38 kinases being the most studied kinases.

1.2.5.1 p38 in Kidney Disease

The p38 mitogen-activated protein kinase (MAPK) is a ubiquitous protein kinase that plays a major role in pro-inflammatory signal transduction pathway in cell response to stress and inflammation. Stimulation of inflammatory cells such as macrophages, neutrophils, and T lymphocytes initiate a cascade of protein phosphorylation leading to phosphorylation of p38 which in turn, it phosphorylates and activates nuclear transcription factors resulting in production of pro-inflammatory cytokines such as IL-6, IL-8, and TNFα that are involved in kidney damage (Zhang et al., 2007).

There are four isoforms of p38: α, β, γ and δ. All of them have a homologous sequence of amino acids but differ in cell and tissue distribution with p38 α, β, and δ isoforms being predominantly present in the kidneys (Wang et al., 1997). In addition, α and β p38 isoforms are found in cells that infiltrate during kidney diseases, such as

macrophages, T cells, and myofibroblasts. Phosphorylation of p38α results in its translocation to the nucleus and the activation of transcription factors involved in production of proinflammatory mediators and extracellular matrix proteins (Stambe et al., 2004). In an *in vivo* study of rat model undergoing surgically induced unilateral uretic ligation and tissue injury, localized p38 MAPK activation was detected within injured tubules and infiltrating myofibroblats of the obstructed kidney.

Another *in vivo* study on autoimmune renal disease highlighted that inhibition of p38 MAPK reduced the severity of the autoimmune disease and prolonged the life span of MRL-Fas lpr mice. Inhibition of p38 MAPK was shown to reduce the infiltration of leucocytes, the expression of cytokines, and the production of immunoglobulins, all of which are known to promote renal injury and result in lethal autoimmune renal injury *in vivo* (Iwata et al., 2003).

1.2.5.2 ERK in Kidney Disease

An important MAPK pathway is the RAS/RAF/MEK/ERK pathway, which in mammals constitutes three RAF proteins (RAF1, A- and B-RAF), two MEK (MEK1 and -2), and two Extracellular signal-regulated protein kinases 1 and 2 (ERK 1/2). The major role of ERK 1/2 has been associated with regulation of cell growth, proliferation, and differentiation (Pearson et al., 2001). ERK 1/2 signaling also contributes to cell survival, apoptosis, morphology determination, and oncogenic transformation (McKay & Morrison, 2007).

Specific protein kinases MAPK/ERK kinases MEK 1 and MEK 2 initiate ERK 1/2 activation. First, Activated RAS stimulates RAF protein kinases RAF1, A-RAF, and B-RAF, which in their turn phosphorylate MEK 1 and MEK 2 (also known as MAP2K1 and MAP2K2). Subsequently, MEK 1 and MEK 2 phosphorylate their substrates, the best-known of which are ERK1/2, which have a wide range of both cytosolic and nuclear targets and functions (Pouysségur & Lenormand, 2016).

ERK signaling has been shown to be activated in compensatory renal hypertrophy, glomerular, and tubulointerstitial diseases. In addition, activation of ERK pathway has

been shown to be involved in polycystic kidney disease while inhibition of the ERK pathway has let to decrease of cyst-induced gain in kidney mass and enhanced renal function (Omori et al., 2006).

1.2.6 Biochemical Markers of Kidney Function

Various biochemical markers can be used to evaluate kidney function. Serum creatinine, urea, and electrolytes are considered markers for routine renal function testing. They provide information about the kidney's glomerular filtration rate (GFR) and tubular function. In addition, the presence of significant amounts of protein in urine is one of the first clinical signs of almost all renal diseases.

Creatinine is a breakdown product of creatine phosphate in muscle produced by the body at a fairly constant rate depending on muscle mass (Zuo et al., 2008). Serum creatinine concentration is a common indicator of kidney function. It is used to calculate glomerular filtration rate, as recommended by the National Kidney Disease Education Program (Miller et al., 2005). The excretion of creatinine by both the glomeruli and the tubules eventually decreases in chronic renal failure and uremia (Edmund & GR, 2006). When serum creatinine levels exceed the upper limit of the "normal" interval, renal failure is usually suspected. However, creatinine levels may fluctuate because its production is influenced not only by muscle mass but also by muscle composition, activity, diet, and health status (Banfi & Del Fabbro, 2006).

Urea is major nitrogenous end product of protein catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. Urea is filtered out of blood by the kidneys and partially reabsorbed with water (Gowda et al., 2010). The concentration of urea in the blood is useful for estimating renal function. In fact, it can help with the differential diagnosis of acute renal failure and pre-renal conditions where the blood urea nitrogen–creatinine ratio is elevated (Rosner & Bolton, 2006). Increased blood urea nitrogen (BUN) levels are associated with several conditions such as kidney disease or failure, nephrolithiasis, congestive heart failure, dehydration, and digestive tract bleeding. But urea clearance is considered as a poor indicator of

glomerular filtration rate because it is affected by non-renal factors like urea cycle enzymes and diet (Pagana, 2014).

Proteinuria estimation aids in distinguishing between tubulointerstitial and glomerular diseases, as well as tracking the progression of renal disease and assessing the response to therapy. Albumin, 2-macroglobulin, IgG, and 2-microglobulin protein panels have been used in the differential diagnosis of pre-renal and post-renal diseases (Tishkov et al., 1978). The protein/creatinine ratio is used as an Index of Quantitative Proteinuria in 24-hour urine collection (Garg et al., 2004). Semi-quantitative dipstick urinalysis is also used for proteinuria evaluation because it is inexpensive and simple to perform (Maybury & Waugh, 2005). However, a dipstick proteinuria finding should be confirmed by a protein-creatinine ratio or a 24-hour urine collection (Waugh et al., 2005).

Electrolyte panel is very often used to test for an electrolyte or acid-base imbalance as well as to monitor the effect of an intervention impacting bodily organ function. An electrolytes test quantifying levels of sodium, potassium, chloride, and bicarbonate is used for the diagnosis and management of renal fibrosis (Dhondup & Qian, 2017). Potassium has been used as a highly reliable electrolyte marker of renal failure. During renal failure, increased plasma potassium is caused by a combination of decreased filtration and decreased potassium secretion in the distal tubule (James & Mitchel, 2006).

1.3 Vanadium Compound

1.3.1 Properties of Vanadium

Vanadium is a group 5d transition metal found in abundance in nature. It was discovered in 1830 by Nils Gabriel Sefström, a Swedish chemist, and is the 18th most abundant element in the Earth's crust, found in soil, water, air, and living organisms (Rehder, 2012). It is listed as one of the 40 essential micronutrients and is required in trace amounts for normal metabolism, growth, and development of mammals (French & Jones, 1993).

1.3.2 Sources of Vanadium

Several dietary sources have been identified as the primary source of vanadium for the human population, despite the fact that vanadium is present in very low concentrations in diets (1 ng/g) (Barceloux, 1999). Vanadium-rich foods include mushrooms, dill seed, parsley, and black pepper, as well as cereals, fresh fruits, and shellfish (Badmaev et al., 1999; Byrne & Kosta, 1978). Vanadium is mostly found in bone, kidney, spleen, liver, lung, and blood, with an intracellular concentration of 20-200 nM (Mongold et al, 1990). Because of the combustion of carbon-based fossil fuels, vanadium compounds are also present in air, soil, and water reservoirs contaminants in large urban agglomerations (Korbecki et al., 2012).

1.3.3 Effects of Sodium Orthovanadate

Vanadium can be transformed into a variety of inorganic compounds, including vanadyl sulfate, sodium metavanadate, sodium orthovanadate, and vanadium pentoxide. Sodium orthovanadate (Na3VO4), also known as vanadate, is a phosphate analog with several biological activities. It inhibits non-selectively protein tyrosine phosphatases (PTPs), alkaline phosphatases, and ATPases. Vanadate also activates tyrosine kinases, promotes mutagenesis and neuroprotection, and inhibits diabetic effects via insulin-mimetic properties (Alonso et al., 2004). In addition, studies have shown that it can slow the growth of tumors in the central nervous system, lung cancer, prostate cancer, bladder cancer, and liver cancer (Yu et al., 2019).

1.3.4 Protein Tyrosine Phosphatase Inhibition

Sodium orthovanadate adopts a trigonal pyramid structure, similarity to phosphate anions, allowing it to fit into the protein tyrosine phosphatases active site and to act as a broad specificity, reversible, and competitive inhibitor of protein tyrosine phosphatase (Irving & Stocker, 2017). Enzyme-bound vanadate moieties are referred to as transition state analogues because the enzyme-vanadate dissociation constants in these enzymes are much lower than those for phosphate (Deng et al., 2002).

In biological systems, vanadium compounds are typically found in +IV or +V oxidation states. Under normal physiological conditions, vanadium in the +IV-oxidation state is present in the form of vanadyl cations (VO^{2+}) and it is present as vanadate ions in the +V-oxidation state, such as orthovanadate ($H_2VO_4^-$) (Crans et al., 2004). When vanadium enters the cytoplasm, it is reduced to +IV-oxidation state by intracellular antioxidants and present as vanadyl cations, resulting in the formation of reactive oxygen species that cause oxidative stress (Ding et al., 1994). The vanadyl cations react with H_2O_2 to form vanadate ions and hydroxyl radicals HO· (Shi & Dalal, 1993).

The generated vanadate enters cells, bind to proteins at cysteine residues, and combine with H_2O_2 to form pervanadate which cause the direct oxidation of cysteine residues that are essential to the enzymatic action of PTPs. Thus, oxidation of PTPs make them catalytically inactive, sometimes irreversibly, hence contributing to broad PTPs inhibition (Huyer et al., 1997; Meng et al., 2013). The reactive oxygen species produced may also inactivate PTPs by the oxidation of cysteine residues in the catalytic centers of these enzymes (Östman et al., 2011).

1.3.5 Vanadium-Induced Nephrotoxicity

Vanadium compounds, in addition to their potential medicinal properties, can cause poisoning. The mechanism of vanadium toxicity is not fully understood and requires additional research. However, it appears that the harmful effects of this metal are linked to oxidative stress, a condition in which cells produce excessive amounts of reactive oxygen species. This can have a variety of negative consequences, including lipid peroxidation, protein denaturation, DNA degradation, and cell membrane disintegration (Wilk et al., 2017).

The urinary system is one of the main excretory routes for vanadate within the body, vanadate accumulation in the kidney causes both structural and functional damage (Barrio & Etcheverry, 2006). It has been reported that proximal tubules are the main site of action of vanadate in the nephron (Higashi and Bello-Reuss, 1980). Several studies documented that vanadate induces more cellular cytotoxicity in the proximal tubules than in the other segments (Boscolo et al., 1994; Soussi et al., 2017).

The pathological effects of vanadium on various kidney structures were demonstrated in several experiments, during which hybrid mice were given samples of a vanadium-rich oil. Renal failure was observed in more than half of the experimental animals, primarily in the form of glomerulonephritis. Moreover, intraperitoneal administration of vanadium to the tested animals caused acute glomerulonephritis with partial tubular and glomerular necrosis, which was associated with acute renal failure (Sarsebekov et al., 1994).

Vanadate administration for 10 day at 5 mg/Kg/day significantly reduced renal 1-Na, K-ATPase protein in both the cortex and the medulla, increased cAMP accumulation, and decreased renal malondialdehyde levels. This resulted in renal histopathological lesions in the glomeruli, particularly the proximal tubules. Furthermore, increases in blood urea nitrogen, plasma creatinine, and fractional excretion of all studied electrolytes were observed as a result of vanadate-induced kidney injury (Eiam-Ong et al., 2018).

One study reported an increase in the kidney/body weight ratio in sodium metavanadate intoxicated rats, implying that vanadate accumulation in the kidney caused significant changes in the size of this organ (Ścibior & Zaporowska, 2007). Furthermore, rats exposed to sodium metavanadate had lower creatinine clearance, higher plasma creatinine and plasma urine levels, and lower urinary creatinine and urine excretion, indicating glomerular functional disorders and problems with creatinine and urine elimination (Ścibior et al., 2014).

Significant age-difference were found in most of the parameters of nephrotoxicity in young (22 days) and adult (62 days) male Sprague-Dawley rats receiving injections of sodium orthovanadate at 10 mg/kg/day for 8 consecutive days. Adverse renal effect and vanadium-induced morphologic changes in the kidney were more pronounced with age (de la Torre et al., 1999).

Furthermore, a significant relationship was found between the dose of vanadium compound and pathological changes in renal tissue. Lesions in the kidneys of rats treated with vanadium at various doses (3, 15, and 30 mg/kg) were observed, where

the kidneys from the last group showed the most significant changes. Granular and vacuolar degeneration were observed in the cells of the renal tubules and the endothelial cells of the glomeruli (Wang et al., 2016).

1.3.6 Detection of Vanadium-Induced Nephrotoxicity

A study found that cystatin C was completely taken up by intact proximal tubular cells in rats, but their exposure to vanadate, which caused tubular disease and proximal convoluted tubule injury, decreased cystatin C uptake and significantly increased cystatin C concentration in the urine. Furthermore, urinary Kidney Injury Marker -1 (KIM-1) excretion was significantly increased due to proximal tubular cell damage after vanadate exposure (Ścibior et al., 2014).

1.3.7 Herbal Medicine in Vanadium-Induced Nephrotoxicity

In the past few years, much interest has been laid on the role of naturally occurring plants for the control and the management of various chronic diseases. It has been shown that formulations derived from plant parts such as flowers and leaves play an important role against vanadium-induced nephrotoxicity. *Malva sylvestris*, for example, has been shown to reduce ammonium metavanadate-induced nephrotoxicity in rats as measured by lipid peroxidation, antioxidant enzyme activities, and histopathological changes (Marouane et al., 2011). Another study found that *Salvia officinalis* sage essential oil has an antioxidant effect against ammonium metavanadate-induced renal toxicity (Koubaa et al., 2019). However, very few studies have attempted to demonstrate cannabis's protective role in kidney disease models.

1.4 Cannabis Oil Extract

1.4.1 Cannabis Sativa

Cannabis is the most commonly used federally illegal drug in the United States (Rein, 2020). It belongs to a group of three plants known as *Cannabis sativa, Cannabis indica, and Cannabis ruderalis*. *Cannabis sativa* L. (*C. sativa*) is an aromatic annual herb that has been widely grown throughout history and harvested for its oil, seeds,

and fiber. It has been used for a variety of medicinal and industrial purposes (Hartsel et al., 2016). Between the eighth and eighteenth centuries, physicians in the Arab world used cannabis and hemp seeds as a traditional herbal remedy (Groom, 2014). Cannabis has anti-inflammatory, anti-emetic, anti-epileptic, and diuretic properties (Clarke and Merlin, 2013). However, its medicinal indications and therapeutic applications are still questioned today.

1.4.2 Cannabis in Lebanon

The most common cannabis strains found in Lebanon nowadays are *Cannabis sativa* and *Cannabis indica*. Cannabis oil has traditionally been used to treat a variety of diseases in Lebanon, including diabetes, chronic pain conditions such as arthritis, and cancer (Mollace et al., 2005). The Lebanese government issued a draft law legalizing cannabis harvesting for medical and industrial purposes on April 22, 2020. (Lebanese Official Gazette; issue 23; 2020).

1.4.3 Cannabis Plant Composition

Cannabinoids, terpenes, and phenolic compounds are the most common phytochemicals found in cannabis plants (Andre et al., 2016). Cannabidiol (CBD) and tetrahydrocannabinol (THC) are the two main components of cannabis oil extract. CBD, a non-psychoactive cannabinoid, has numerous pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial, anxiolytic, and anticonvulsant properties (Sangiovanni et al., 2019). THC, a psychoactive cannabinoid, is primarily used to treat pain, cancer, multiple sclerosis, and neurodegenerative disorders (Koppel et al., 2014).

It is important to note that combining CBD and THC boosts their therapeutic activity (Russo & Guy, 2006). Besides that, the presence of other plant constituents like terpenes and phenols with CBD improves its therapeutic effect. This is referred to as the 'entourage effect,' and it has been studied in chemically induced intestinal inflammation as well as inflammatory murine models of Huntington and Alzheimer diseases (Gallily & Yekhtin, 2019; Pagano et al., 2016).

1.4.4 The Endocannabinoid System

Normal renal physiology is influenced by the endocannabinoid (EC) system. In fact, significant cannabinoid receptors and machinery have been found in kidney tissues (Kondo et al., 1998; Ritter et al., 2016). Constituents of the EC system include endogenous fatty acid-derived ligands, their receptors, and the enzymes required for their biosynthesis and degradation (Howlett, 2002). The two well-identified ECs are 2-arachidonoyl-*sn*-glycerol (2-AG) and *N*-arachidonoyl ethanolamide, also known as anandamide (AEA).

Endocannabinoids act on cells by interacting with cannabinoid receptors, specifically cannabinoid subtypes 1 (CB_1) and 2 (CB_2) (Munro et al., 1993). Functional CB_1 receptors are present in proximal convoluted tubules, distal tubules, and intercalated cells of the collecting duct in the human kidneys (Larrinaga et al., 2010). CB_1 receptors expression is localized in afferent and afferent arterioles (Koura et al., 2004), thick ascending limbs of the loop of Henle (Silva et al., 2013), and glomeruli of rodents (Barutta et al., 2010). CB_2 receptors were found in podocytes (Barutta et al., 2010), proximal tubule cells (Jenkin et al., 2010), and mesangial cells of human and rats (Deutsch et al., 1997).

1.4.5 Role of Endocannabinoid System in Nephrotoxicity

The potential role of EC system in treating nephrotoxicity has been an emerging area of research, specifically in the context of cannabinoid receptors. Alterations of cannabinoid receptors have been involved in different renal disease such as acute kidney injury, chronic kidney disease, and diabetic nephropathy. In mice kidney model, the selective CB_1 and CB_2 receptor agonists showed a dose-dependent effect in preventing tubular damage after renal ischemia-reperfusion (Feizi et al., 2008). Moreover, cannabidiol decreased renal tubular injury in rats following bilateral renal ischemia/reperfusion by preventing increase in serum creatinine, nitric oxide, and renal malondialdehyde levels (Fouad et al., 2012). It was also revealed that post-injury treatment with CBD decreased kidney oxidative damage and inflammation in an ischemia-reperfusion animal model where decreased renal myeloperoxidase activity was shown in CBD- treated animal group (Soares et al., 2015).

1.5 Hypothesis and Objectives

1.5.1 Hypothesis

Lebanese cannabis oil extract may demonstrate significant therapeutic effect against sodium orthovanadate-induced nephrotoxicity in rats.

1.5.2 Objectives

To evaluate the *in vivo* therapeutic effect of Lebanese cannabis oil extract against sodium orthovanadate-induced nephrotoxicity by the assessment of:

- Biochemical parameters:
  - Serum Creatinine Level
  - Serum Urea Level
  - Electrolytes Levels
- Histopathological examination of the kidneys

CHAPTER TWO

METHODS

2.1 Chemicals, Materials, and Equipment

The following solutions were prepared for rats' injections:

- Vehicle solution was prepared by mixing Absolute Ethanol, Tween 80, and Phosphate-Buffered Saline (PBS) at a ratio of 1:1:18.

- Sodium orthovanadate was purchased from Sigma-Aldrich (St. Louis, USA). Sodium orthovanadate was dissolved in distilled water at the concentration of 10 mg/mL just before the experiment.

- Dried samples of Cannabis sativa L. ssp. indica were provided to us by the Drug Enforcement Office in Zahle, Beqaa Governorate. Cannabis plant material was securely stored in a special storage facility at the Lebanese American University Laboratory. According to Shebaby et al. (2021), approximately 1.2 g of cannabis oil was extracted from 10 g of air-dried cannabis flowers. Cannabis oil extraction was performed using ethanol for 48 hours. The obtained natural extract was then filtered and concentrated at 45 degrees Celsius under reduced pressure to yield pure cannabis oil extract.

2.2 Animals

Sprague Dawley male rats, aged 8 weeks, were obtained from the animal facility at the Lebanese American University following Animal Care and Use Committee, and were housed under controlled conditions. Food and water were provided ad libitum. National Institutes of Health guidelines were followed for animal care and handling. All efforts were made to minimize animal suffering and reduce the number of animals used.

2.3 Experimental Protocol

Rats were randomly divided into five groups as the following:

- **Group 1: Control** (n = 8)
  Injected with 0.2 mL of intraperitoneal vehicle solution consisting of Ethanol: Tween 80: PBS (1:1:18) daily for 10 days.

- **Group 2: Sodium orthovanadate** (n = 8)
  Injected with 10 mg/Kg intraperitoneal sodium orthovanadate daily for 10 days.

- **Group 3: Sodium orthovanadate + COE (5 mg/Kg)** (n = 8)
  Injected with 10 mg/Kg intraperitoneal sodium orthovanadate daily for 10 days.
  5 mg/Kg intraperitoneal cannabis oil extract was administered starting day 4 till day 10.

- **Group 4: Sodium orthovanadate + COE (10 mg/Kg)** (n = 8)
  Injected with 10 mg/Kg intraperitoneal sodium orthovanadate daily for 10 days.
  10 mg/Kg intraperitoneal cannabis oil extract was administered starting day 4 till day 10.

- **Group 5: Sodium orthovanadate + COE (20 mg/Kg)** (n = 8)
  Injected with 10 mg/Kg intraperitoneal sodium orthovanadate daily for 10 days.
  20 mg/Kg intraperitoneal cannabis oil extract was administered starting day 4 till day 10.

All animals were weighed on days 1, 3, 5, 7, 9 and 11. Rats were scarified on the day 11 of the experiment.

2.4 Biochemical Analysis

2.4.1 Serum Analysis

Blood samples were collected immediately after the sacrifice of rats. EDTA was added to prevent clotting of the samples in a ratio of 25 µL for each 1 mL of blood collected. Then, blood samples collected were centrifuged at 3000 rpm for 20 min at 4◦C for efficient separation and recovery of plasma. The concentrations of the renal function biological parameters including serum creatinine, urea, and electrolytes were analyzed.

2.5 Histopathologic Testing

The kidneys and hearts of all groups were dissected out and weighed. Relative kidney weights were measured by dividing the kidney weight (g) to the rat body weight (g) in all groups. Relative heart weights were measured by dividing the heart weight (g) to the rat body weight (g) in all groups. The organs were fixed in 10% neutral buffered formalin. Then, they were taken, dehydrated and embedded in paraffin. Tissue sections from the paraffin-embedded blocks were mounted on glass slides, deparaffinized, rehydrated, stained with Hematoxylin & Eosin and then examined microscopically.

2.6 Statistical Analysis

All data were reported as plus or minus standard error (SE). The groups were compared using one-way analysis of variance (ANOVA) and the level of statistical significance was set at $P < 0.05$. The analyses were performed using IBM SPSS Statistics Version 26.

CHAPTER THREE

RESULTS

3.1 Evaluation of Body, Absolute and Relative Organ Weights

Table 1 - Body, absolute, and relative organs weights of control and rats treated with sodium orthovanadate and cannabis oil extract.

	Control (Vehicle)	**Sodium orthovanadate**	**Sodium orthovanadate + COE (5mg/Kg)**	**Sodium orthovanadate + COE (10mg/Kg)**	**Sodium orthovanadate + COE (20mg/Kg)**
Body Weight Gain (g)	85.50±1.73	58.38±5.32*****	52.57±4.36	51.88±3.72	51.43±5.47
Absolute Kidney Weight (g)	0.81±0.03	0.80±0.02	0.72±0.03	0.82±0.03	0.72±0.02^{+}
Relative Kidney Weight (g)	0.42±0.01	0.45±0.02*	0.45±0.01	0.47±0.01	0.45±0.01
Absolute Heart Weight (g)	0.74±0.02	0.64±0.03*	0.58±0.03	0.66±0.03	0.59±0.04
Relative Heart Weight (g)	0.39±0.01	0.36±0.01	0.37±0.01	0.38±0.01	0.37±0.03

Values are means ± SE for eight rats in each group.

Sodium orthovanadate group vs Control group: *P-value < 0.05

Sodium orthovanadate + COE groups vs Sodium orthovanadate group: $^{+}$P-value < 0.05

Table 1 shows body weight gain from day 1 till day 11 of experiment, in addition to absolute, and relative kidney and heart weights. A significant decrease in body weight gain was observed in the group of rats taking sodium orthovanadate at a dose of 10 mg/Kg daily for 10 days in comparison with its respective control group. However, the body weights of rats taking sodium orthovanadate and treated with cannabis oil

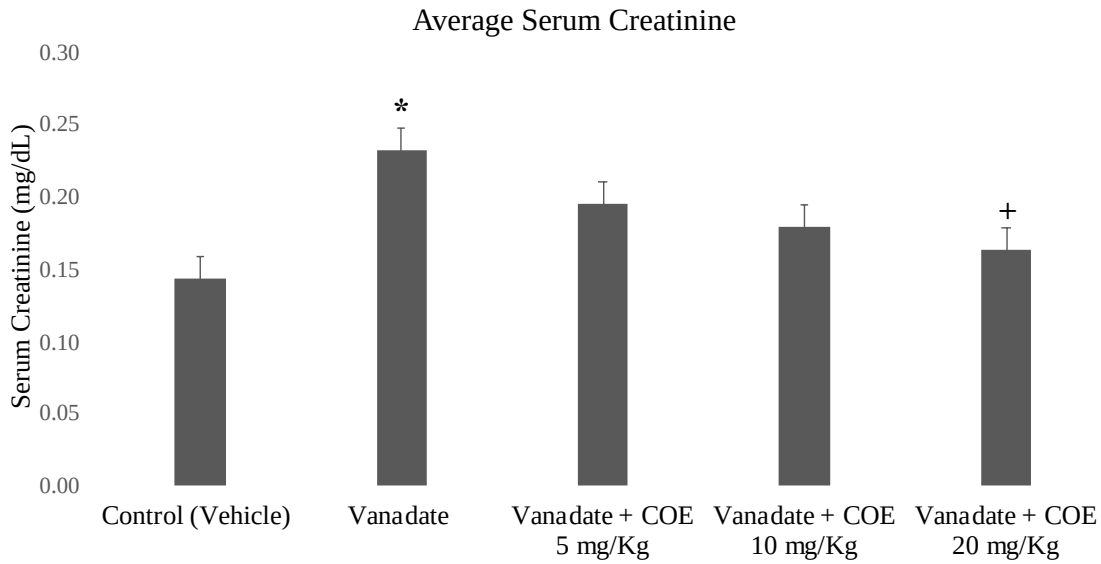
extract at different doses (5, 10, and 20 mg/Kg daily for 7 days) were not different from those of rats taking sodium orthovanadate only.

The results in table 1 show significant decrease in absolute kidney weight of rats taking sodium orthovanadate 10 mg/Kg and cannabis oil extract 20 mg/Kg when compared to sodium orthovanadate group. There is also a significant increase in the relative kidney weight of rats taking sodium orthovanadate 10 mg/Kg daily for 10 days when compared to control rats.

In addition, absolute heart weight of rats taking sodium orthovanadate 10 mg/Kg daily for 10 days was significantly lower than control group. And there were no significant differences in relative heart weight between rats taking sodium orthovanadate and cannabis oil extract at different doses and rats taking sodium orthovanadate only.

3.2 Evaluation of Renal Biomarkers

3.2.1 Average Serum Creatinine Levels



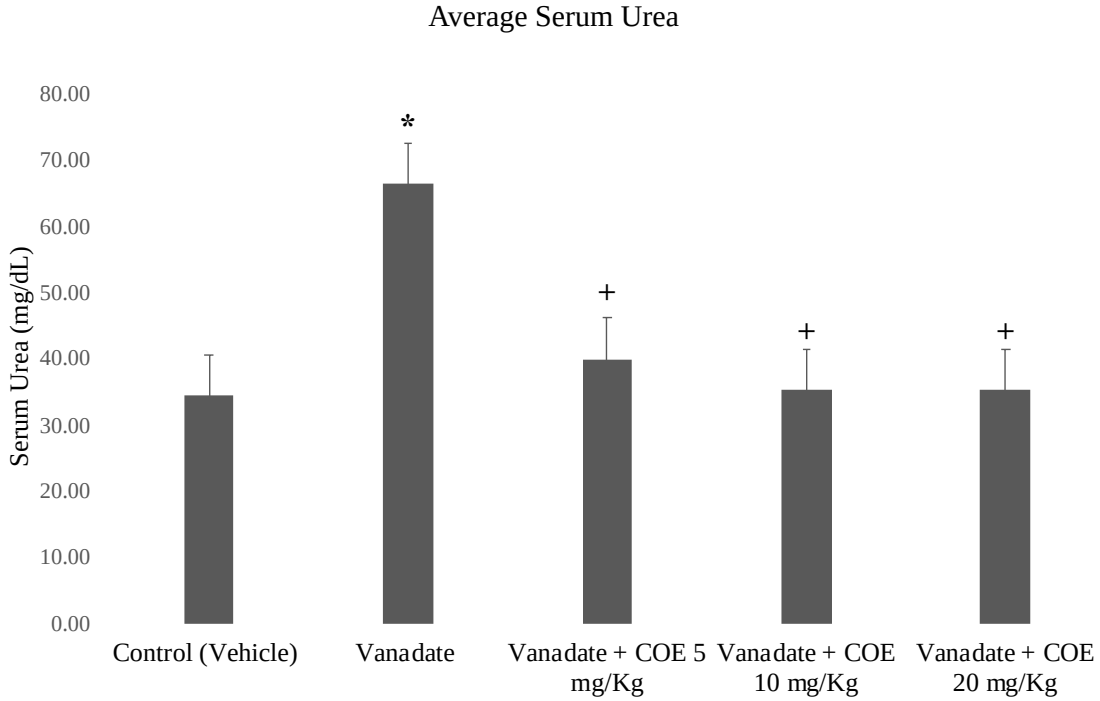
Values are means ± SE for eight rats in each group.
Sodium orthovanadate group vs Control group: *P-value < 0.05.
Sodium orthovanadate + COE groups vs Sodium orthovanadate group: $^{+}$P-value < 0.05.

Figure 1 - Average Serum Creatinine Levels of control and rats treated with sodium orthovanadate and cannabis oil extract.

Levels of serum creatinine were measured to investigate the effect of cannabis oil extract on sodium orthovanadate-induced renal toxicity in rat models. As seen in figure 1, an intraperitoneal injection of sodium orthovanadate at a dose of 10 mg/Kg daily for 10 days lead to a significant increase in serum creatinine levels when compared to the control group ($P < 0.05$). No rats were dead before the assigned sacrifice day of the experiment.

In addition, treatment with cannabis oil extract at a dose of 20 mg/Kg for 7 days lead to a significant decrease in serum creatinine levels when compared to sodium orthovanadate group ($P < 0.05$). However, treatment of rats with cannabis oil extract at lower doses, 5 mg/Kg and 10 mg/Kg for 7 days, did not show significant differences in serum creatinine levels when compared to sodium orthovanadate group ($P < 0.05$).

3.2.2 Average Serum Urea Levels



Values are means ± SE for eight rats in each group.
Sodium orthovanadate group vs Control group: *P-value < 0.05.
Sodium orthovanadate + COE groups vs Sodium orthovanadate group: $^{+}$P-value < 0.05.

Figure 2 - Average Serum Urea Levels of control and rats treated with sodium orthovanadate and cannabis oil extract.

Levels of serum urea were also measured to investigate the effect of cannabis oil extract on sodium orthovanadate-induced renal toxicity in rat models. As seen in figure 2, an intraperitoneal injection of sodium orthovanadate at a dose of 10 mg/Kg daily for 10 days lead to a significant increase in serum urea levels when compared to the control group ($P < 0.05$). Treatment with cannabis oil extract at a dose of 5 mg/Kg for 7 days lead to a significant decrease in serum urea levels when compared to sodium orthovanadate group ($P < 0.05$). In addition, treatment with cannabis oil extract at a dose of 10 mg/Kg for 7 days showed a significant decrease in serum urea levels when compared to sodium orthovanadate group ($P < 0.05$). Furthermore, treatment with cannabis oil extract at a dose of 20 mg/Kg for 7 days showed a significant decrease in serum urea levels when compared to sodium orthovanadate group ($P < 0.05$).

3.2.3 Average Serum Electrolytes Levels

Table 2 - Average Serum Sodium, Potassium, and Chloride Levels of control and rats treated with sodium orthovanadate and cannabis oil extract.

	Control (Vehicle)	**Sodium orthovanadate**	**Sodium orthovanadate + COE (5mg/Kg)**	**Sodium orthovanadate + COE (10mg/Kg)**	**Sodium orthovanadate + COE (20mg/Kg)**
Na+ (mmol/L)	135.63±1.21	137.±1.21	138.86±0.67	138.75±1.63	139±1.59
K+ (mmol/L)	5.19±0.45	5.29±0.34	5.68±0.47	6.59±0.56	6.28±0.72
Cl- (mmol/L)	94.50±0.65	95.89±0.95	95.86±1.32	95.56±0.49	97.90±0.55

Values are means ± SE for eight rats in each group.

The serum concentrations of sodium, potassium, and chloride are presented in table 2. There were no significant differences in average serum sodium, potassium, and chloride levels between rats taking sodium orthovanadate only and control group. Also, there were no significant differences in average electrolytes levels between rats taking sodium orthovanadate and cannabis oil extract and rats taking sodium orthovanadate only.

3.3 Histopathological Findings of Renal Tissue

In the control group, there were no specific pathological deteriorations or changes in glomeruli, tubules, interstitial tissues and/or peritubular capillaries in Hematoxylin & Eosin staining.

It was evident that the injection of sodium orthovanadate at a dose of 10 mg/Kg daily for 10 days evoked marked pathological differences compared with vehicle solution in 40% of tested samples of rats injected with sodium orthovanadate only. Microscopic views of the sodium orthovanadate treated rats revealed acute tubular injuries including vascular dilatation and scattered foci of acute tubular necrosis. In addition, numerous mitosis in tubular cells were observed.

Rats that were given sodium orthovanadate and cannabis oil at doses 5mg/Kg and 20mg/Kg showed normal architecture of glomeruli and tubules; no inflammation, cysts, crystals, glomerular collapse, or deposition were found.

However, cannabis oil treatment at dose of 10mg/ Kg could not ameliorate the destructive pathological properties induced with sodium orthovanadate as vascular dilatation were observed in 50% of rats injected with sodium orthovanadate and cannabis oil extract at a dose of 10 mg/Kg.

There were no significant findings (inflammation, deposition, and myocyte injury) in the heart tissues of all rats except mild chronic inflammation was observed in a rat that was treated with sodium orthovanadate and cannabis oil extract at dose 10mg/Kg.

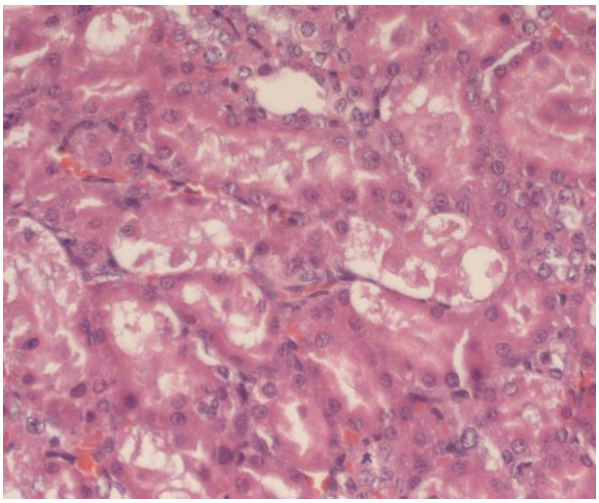


Figure 3 - Micrograph of renal sections from sodium orthovanadate treated rats revealing scattered foci of ATN.

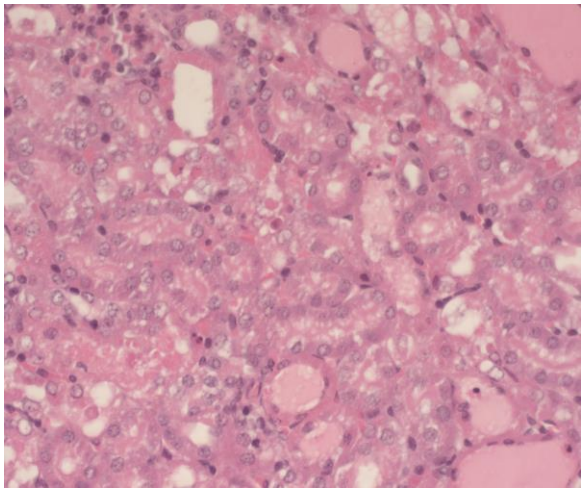


Figure 4 - Micrograph of renal sections from sodium orthovanadate treated rats revealing minimal vascular dilation.

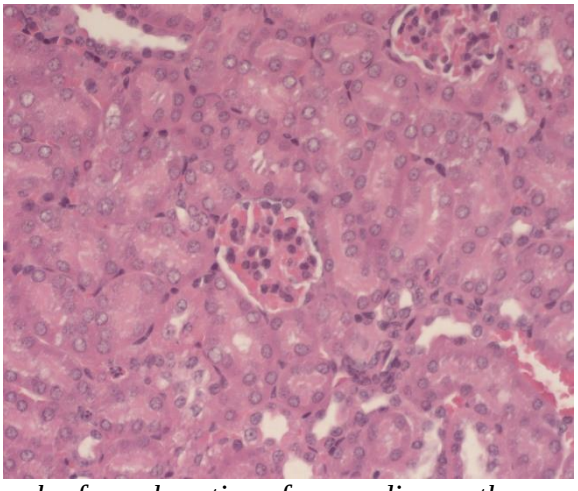


Figure 5 - Micrograph of renal sections from sodium orthovanadate and 10mg/Kg COE treated rats.

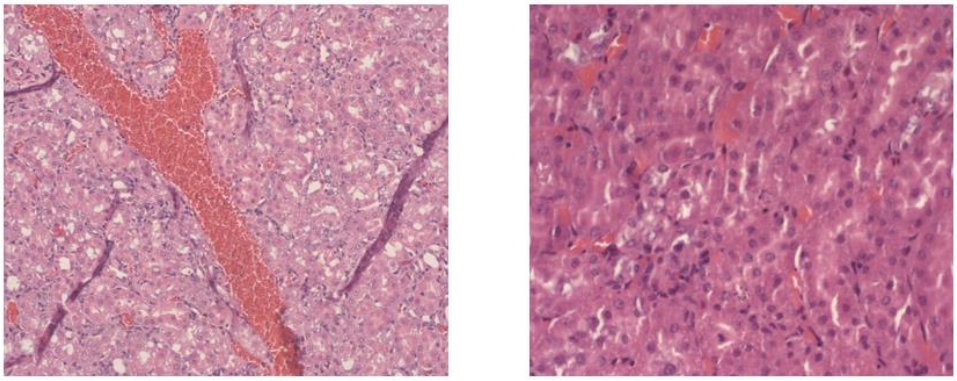


Figure 6 - Micrographs of heart sections from sodium orthovanadate treated rat.

CHAPTER FOUR
DISCUSSION

Vanadate, an oxyanion derivative of vanadium, can be found in soil, water, air, plants, animals, and human tissue in varying concentrations (Rehder, 2015). The urinary system is one of the main excretory routes for vanadate within the body (Barrio & Etcheverry, 2006). Hence, the kidney is especially vulnerable to the negative effects of vanadate. The renal system susceptibility to vanadate may be due to its accumulation in this tissue (Donaldson et al., 1985). It has been shown that vanadate causes greater cellular cytotoxicity in the proximal tubules than in the other segments (Soussi et al., 2017).

In recent years, there has been a lot of focus on the role of naturally occurring plants in the control and management of various chronic diseases. Several studies have been conducted to investigate the anti-vanadium-induced toxicity effects of formulations derived from plant parts like flowers and leaves (Marouane et al., 2011; Koubaa et al., 2019). However, there have been very few studies that have attempted to demonstrate cannabis's treatment role in kidney disease models.

The most common cannabis strains found in Lebanon nowadays are *Cannabis sativa* and *Cannabis indica*. Cannabis oil has traditionally been used to treat a variety of diseases in Lebanon, including diabetes, chronic pain conditions such as arthritis, and inflammation (Shebaby et al., 2021). The potential role of the endocannabinoid system in treating nephrotoxicity is a new area of study, particularly in the context of cannabinoid receptors. Alterations of cannabinoid receptors have been linked to different renal diseases, including acute kidney injury, chronic kidney disease, and diabetic nephropathy (Park et al., 2017). The selective CB_1 and CB_2 receptor agonists showed a dose-dependent effect in preventing tubular damage after renal ischemia-reperfusion in a mouse kidney model (Feizi et al., 2008).

Rats that were given sodium orthovanadate at a dose of 10 mg/kg daily for 10 days had a significant decrease in body weight gain in comparison to the control group (58.38±5.32 versus 85.50±1.73). These data were in agreement with previous studies

reporting the significant body weight decrease following vanadate administration in rats (de la Torre et al., 1999; Wilk et al., 2017). In addition, there was a significant increase in rats' relative kidney weight after taking sodium orthovanadate 10 mg/Kg daily for 10 days when compared to control rats (0.45±0.02 versus 0.42±0.01). Previous study revealed an increase in rat kidney weight after administration of vanadium compound (Koubaa et al., 2019).

In addition, absolute heart weight of rats taking sodium orthovanadate 10 mg/Kg daily for 10 days was significantly lower than control group (0.64±0.03 versus 0.74±0.02). The decrease in heart muscle mass may be associated with decreased vascularization and perfusion to the nephrons. As a result, prerenal nephrotoxicity occurs leading to a decrease in the GFR. Fundamentally, it is related to an imbalance in the delivery of nutrition and oxygen to the nephrons during periods of increased energy demand. Therefore, any process that affects the systemic circulation or decreases renal perfusion can compromise the GFR and kidney function (Manzoor & Bhatt 2020).

A rise in serum creatinine levels of rats taking sodium orthovanadate was observed when compared with the control (0.23±0.08 versus 0.14±0.03) indicating renal injury. A decline in serum creatinine levels was detected in rats taking sodium orthovanadate and cannabis oil extract at a dose of 20mg/Kg when compared to rats taking sodium orthovanadate only (0.16±0.03 versus 0.23±0.08).

Moreover, the administration of sodium orthovanadate lead to an increase in serum urea levels compared with the control (66.50±28.83 versus 34.38±4.68) implying nephrotoxicity. An increase in plasma levels of creatinine and urea in vanadium-treated rats when compared with the control group was observed in previous study (Eiam-Ong et al., 2018). Theoretically, modifications in the metabolism of urea could be linked to a decrease in muscle mass. Given that muscle tissue has a high protein content and that urea is the ultimate catabolite of endogenous protein breakdown, it is possible that a loss in muscle mass has resulted in an increase in urea production (Bankir 1996).

Promising findings observed were the decrease in serum urea levels of rats taking sodium orthovanadate and cannabis oil extract at different doses (5mg/Kg; 10 mg/Kg; 20mg/Kg) when compared to the group of rats taking sodium orthovanadate only

(40±14.31; 32.25±6.14; 35.29±10.95 respectively versus 66.50±28.83). In fact, a previous study conducted on mice investigated the nephroprotective effect of cannabidiol revealing a decrease in serum creatinine and urea when administered at doses of 5 mg/Kg and 10 mg/Kg (Pan et al., 2009). Our findings showed that the administration of cannabis oil extract at a dose of 20 mg/kg could also play an important role in kidney function repair, which could be a promising approach to protect against sodium orthovanadate-induced nephrotoxicity.

A better biomarker of kidney injury is one that the kidneys directly release into the blood or urine in response to the injury. Additional test that might be used to evaluated renal toxicities include urinary markers kidney injury molecule-1 (KIM-1), 2-microglobulin, cystatin C, clusterin, and trefoil factor-3 have been recognized as highly sensitive and specific urinary biomarkers to monitor drug-induced kidney injury in preclinical studies by the Food and Drug Administration (van Meer et al., 2014).

These outcomes indicate that cannabis oil extract provides significant protection against sodium orthovanadate induced nephrotoxicity at different doses. However, there were no clear changes in serum sodium, potassium, and chloride levels between the different groups, which were in agreement with the previous experiment in rats demonstrating renal toxicities induced by vanadium compound (Zendeboodi et al., 2019). The lack of effect on electrolytes levels can be explained to the lack of extensive damage to the kidneys, which allowed renal compensation. In fact, homeostasis of calcium and phosphate, electrolyte and fluid balance, and acid-base balance are all important functions of the kidneys. The ability of the kidney to carry out these functions is compromised when nephron number and GFR decline, and compensatory or alternative mechanisms are generated to partially normalize kidney function and the body's excretory capacity impacting the glomeruli and the tubular system (Isakova et al., 2011; Ueda et al., 2016; Layton et al., 2018)

The administration of sodium orthovanadate at a dose of 10 mg/Kg daily for 10 days evoked marked major signs of renal nephrotoxicity including vascular dilatation, scattered foci of acute tubular necrosis, and numerous mitosis in tubular cells. Previous studies reported that vanadium, as many other metals, tend to accumulate in the kidney predisposing to nephrotoxicity (Al- Bayati et al., 2002; Marouane et al., 2011). It has

also been reported that the kidney is vulnerable to damage as a result of increased perfusion and the increased concentration of excreted compounds in renal tubular cells (Mohamed et al., 2003).

Cannabis oil treatment at dose of 10mg/Kg could not decrease the damaging pathological properties induced with sodium orthovanadate as vascular dilatation were observed in the 50% of rats injected with sodium orthovanadate and cannabis oil extract at a dose of 10 mg/Kg. However, rats that were taking sodium orthovanadate and cannabis oil at doses 5mg/Kg and 20mg/Kg showed normal architecture of glomeruli and tubules; no inflammation, cysts, crystals, glomerular collapse, or deposition were found. These histological studies revealed that the pathological lesions induced by sodium orthovanadate were remarkably reduced by the administration of cannabis oil extract at doses of 5mg/Kg and 20mg/Kg which were in agreement with the results of renal functional biomarkers including serum creatinine levels and serum urea levels.

The initiation and extension phases of AKI are significantly influenced by inflammation. In ischemia, sepsis, and nephrotoxic models, studies have suggested that the first insult causes alterations in the morphology and/or functionality of vascular endothelial cells and/or tubular epithelium. The damaged kidneys are then invaded by leukocytes such as neutrophils, macrophages, natural killer cells, and lymphocytes. The injury induces the generation of inflammatory mediators like cytokines such as TNF, IL-6, and IL-18 by tubular and endothelial cells which contribute to the recruiting of leukocytes into the kidneys (Akcay & Edelstein, 2009).

The mechanisms by which the cannabinoid receptors modulate or recover tubular cell survival following acute damage are not yet well defined till today. However, the possible underlying mechanisms in improving kidney structure and function in this study include antagonism of the CB_1 receptor and/or activation of the CB_2 receptor.

THC acts a partial agonist at the CB_1 and CB_2 receptors (Pertwee, 2008) whereas CBD acts as CB_1 antagonist and as a negative allosteric modulator at CB_2 receptors (Levinsohn & Hill, 2020). The blockade of the CB_1 receptor and/or activation of the CB_2 receptor was shown to be protective against tubular damage by attenuating renal oxidative stress and inflammation (Mukhopadhyay et al., 2016).

A previous study revealed that possible pathways underlying cannabis activity include activation of the CB_2 receptor causing decreased infiltration of immune cells, specifically leukocytes, into the kidney and attenuating inflammatory cytokine release and therefore its anti-inflammatory effect (Mukhopadhyay et al., 2010).

Moreover, it has been revealed that in animal models of acute kidney injury, CB_1 receptor activation is associated with increased production of reactive oxygen species, which can activate the transcription of downstream proinflammatory target genes leading to the activation of programmed cell death by apoptosis (Park et al., 2017). Hence, cannabis oil administration may have played a role in inhibiting CB_1 receptor activation and blocking the production of inflammatory proteins and therefore decreasing cell injury and death.

CHAPTER FIVE
CONCLUSION

The current study investigated the protective effect of Lebanese cannabis oil Extract against Sodium orthovanadate-induced nephrotoxicity. Rats that were injected with Sodium orthovanadate displayed a marked reduction in body weight, increase in serum creatinine and urea in comparison to the control group. All doses of Cannabis oil caused significant decrease in serum urea, as well as in serum creatinine at a dose of 20mg/Kg. In addition, a marked reduction in renal vascular dilatation, scattered foci of acute tubular necrosis, and numerous mitosis in tubular cells was observed in Cannabis oil treated rats (20mg/Kg) as compared to the Sodium orthovanadate- treated group. In conclusion, the primary findings demonstrate a potential therapeutic effect of Cannabis oil on kidney damage induced by Sodium orthovanadate. Further studies are needed to confirm these findings and to investigate the signaling pathways underlying the protective effect of cannabis oil extract against sodium orthovanadate-induced renal toxicity.

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