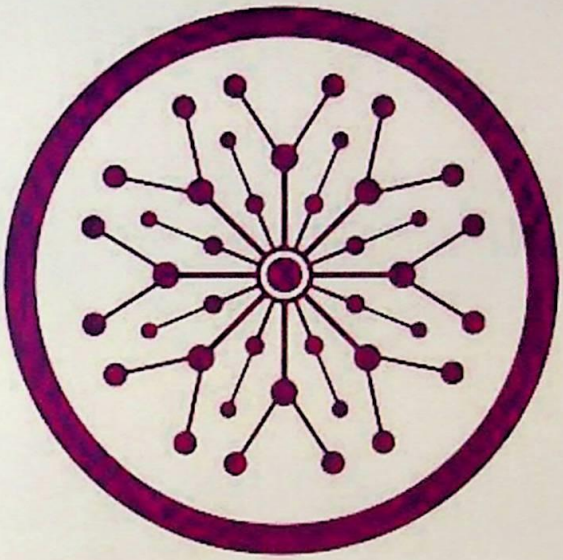
EVALUTION OF ANTIUROLITHIC EFFECT OF STATIN AND BETA CAROTENE COMBINATION IN RODENTS MODEL



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THE SUPERIOR UNIVERSITY, LAHORE

In Partial Fulfillment of the

Requirement for the Degree of

Master of Philosophy in Pharmacology

By

Kiran Fatima

Roll No: MSPC-F21-013

Session: 2021-2023

FACULTY OF PHARMACY

SUPERIOR UNIVERSITY, LAHORE


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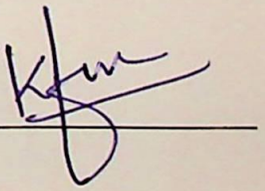
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Dated: May, 20, 2023

SUBJECT: ETHICAL APPROVAL CERTIFICATE

This is to certify that the research project titled "*Evaluation of antiurolithic effect of statin and beta carotene combination in rodents*" conducted by Kiran Fatima under the supervision of Dr. Shafiq Ali Shah at Faculty of Pharmacy, Superior University Lahore, Pakistan has been reviewed and approved by the Ethics Committee.

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1. You are advised to follow the WHO guidelines while conducting the research. In case you feel unusual, you must report to the healthcare department within 24 hours.
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No Part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the Faculty of Pharmacy, Superior University, Lahore in partial fulfilment of the requirements for the degree of Master of Philosophy in field of **Pharmacology** in Faculty of Pharmacy at Superior University, Lahore.

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DEDICATION

To Allah Almighty, I am thankful to you for giving me all these blessings, and then recount them individually as many as you can.

To my all Teachers, for their guidance, support and motivation for work. They always give me a right path and strengthen my Knowledge with positive energy. I dedicate my thesis with special thanks and respect to my all mentors including Dean Shb, HOD Shb, Supervisor, Co- Supervisor and remaining all my teachers they support throughout my academic career including Ist grade to M.phil study.

To my Parents, for their unconditional Love, endless encouragement, support throughout my life and especially during my academic pursuits. Their belief in me has my guiding light and I dedicate this thesis to them with all my heart.

To my Friends, this dedication is specially to my all kind of support Dr Ameer Hamza, Dr Muhammad Muzammil, Muhammad Sultan Salhuddin and best friends like sister Sidra Rafique, Aleesha Iqbal & Mishal Fatima who always stand beyond me throught out my academic career and especially in my Research and thesis work. They always support me and give me a positive energy for my work. Also dedicate to all my friends and colleague they support me in my thesis. I dedicate this thesis to them from my bottom of my Heart.

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ABSTRACT

Urolithiasis is one of the oldest known diseases affecting humans across different cultures and time periods. The precise pathophysiology of Urolithiasis has yet to be elucidated. Kidney Stone formation is a complex that results from a succession of several physicochemical events including super saturation, nucleation, growth, aggregation and retention within the kidneys. Urinary stones affect 10-12 % of the population in industrialized countries Urinary stone disease continues to occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10 %. A predominance of men over women can be observed with an incidence peak between the fourth and fifth decade of life. Urolithiasis is one of the major diseases of the urinary tract and is a major source of morbidity. Stone formation is one of the painful urologic disorders that occur in approximately 12% of the global population and its re-occurrence rate in males is 70-81% and 47-60% in female. 1 It is assessed that at least 10% of the population in industrialized part of the world are suffering with the problem of urinary stone formation. its annual incidences are increasing and also the age of onset is decreasing, perhaps due to change in life styles, diet and climate. Improvement in therapy of kidney stones with modern techniques like extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), and percutaneous nephrolithotomy (PNL), has revolutionized the urological practice but the recurrence in kidney stone is not altered. ESWL is less effective in calcium oxalate monohydrate (COM) and cystine stones than calcium oxalate dihydrate (COD) and uric acid stones . In addition to the high cast and the issues of recurrence with these measures there are multiple side effects, which include renal damage, ESWL induced hypertension, renal impairment, sever hematuria, steinstrasse (multiple small stone blocking ureter), pancreatitis,

infection and persistent residual fragments as potential nidus for new stone formation. While these complications can lead to large perfusion of the collecting system, extravasations of irrigating fluid, urosepsis, ureteral injury and delayed bleeding may also occur. Pharmacological agents include a limited choice like citrate and thiazide diuretics, which have limited efficacy in addition to their less tolerability.

Keywords: Urolithiasis, Physicochemical, Predominance, Calcium oxalate, Perfusion

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LIST OF ABBREVIATION AND ACRONYMS

At	Atorvastatin
AD	Alzheimers disease
AAPH	2-amino-propane hydrochloride
BC	Beta Carotene
BSA	Bovine serum albumin
CAT	Catalase activity
CARET	Carotene and retinol efficacy trials
COD	Calcium oxalate dihydrate
COM	Calcium oxalate monohydrate
CKD	Chronic kidney disease
CVD	Cardiovascular Disease
ESWL	Extracarporeal shock wave lithotripsy
eNOS	Endothelial nitric oxide synthase
GFR	Glomerular filtration rate
HMG	Hydroxy methyl glutryl
IP	Intra peritoneal
LDH	Lactate dehydrogenase
ORAC	Oxygen radical absorbance capacity
PNL	Percutaneous nephrolithotomy
RA	Retinoic acid
ROS	Reactive oxygen species
SOD	Sodium dismutase
TNF	Tumor necrosis factor

CHAPTER: 1

INTRODUCTION

1.INTRODUCTION

1.1 Urolithiasis

Urolithiasis, development of the urinary tract stone, is among the earliest illnesses recognized.. Significant proof that kidney and bladder stones have plagued people for ages can be discovered in archeological discoveries; research on Egyptian mummies has also identified kidney and bladder stones [1, it is the third most prevalent urinary tract issue. A high recurrence rate of around 10–23% annually, 50% within 5–10 years, and 75% within 20 years is linked to it. Relapses become more frequent and their intervals get shorter as the illness gets worse. [1]) Furthermore, there is a decrease in the age of onset and an increase in the annual incidence, which may be brought on by alterations in climate, diet, and lifestyle[2].

Despite improvements in kidney stone therapy with modern therapies, the incidence of kidney stone recurrences has not decreased. Regarding uric acid and calcium oxalate dihydrate (COD) stones, ESWL is more efficient than calcium oxalate monohydrate (COM) and cystine stones [3]. These procedures have several side effects, including severe hematuria, kidney damage, ESWL-induced hypertension, renal failure, pancreatitis, infection, and permanent obstruction of the ureter, in addition to their high likelihood of recurrence. The residue is crushed, if at all possible, to make Lavage fluid extravasations, ureteral damage, profuse perfusion of the collecting system, and late bleeding are possible outcomes of these problems . Pharmacological medicines come in a narrow spectrum; examples are thiazide diuretics and citrate diuretics, which have inferior tolerability and limited efficacy. Urolithiasis is formation of urinary tract stone, which includes the kidneys, urethra, bladder, and urethra. "Lithos" (stone) and ouron (urine) make up "urolithiasis".

1.2 Epidemiology

Urinary stone disease is one of the most prevalent urinary tract conditions and a major cause of morbidity. About 12% of people worldwide suffer from stone formation, one of the most painful urological disorders. In women, the recurrence rate is 47–60%, while in men it is 70–81%. 1. It is estimated that at least 10% of people in the industrialized world struggle with the development of urinary stones.Compared to other regions, the southern region has a lower incidence of kidney stones. Urinary stone disease is one of the most prevalent urinary system conditions and a obvious cause of morbidness. About 12% of people worldwide suffer from kidney stone, one of the most critical urological disease. In women, the recurrence rate is 47–60%, while in men it is 70–81%. 1. It is estimated that at least 10% of people in the industrialized world struggle with the development of urinary stones.Kidney stones are less common in the south than in other parts of the country[4] .Because it boosts testosterone and inhibits estrogen's ability to create stones, the effect is three times greater in males than in women. various urolithiasis kinds The mineral content of the stone determines its name. The most prevalent types of stones are silica, calcium oxalate, urate, struvite, and cysteine [5]

1.3 Types of Kidney Stone

It is more common in man as compared to woman Because it raises testosterone and decreases estrogen's ability to create stones, the effect is three times stronger in males than in women. various urolithiasis kinds The mineral content of the stone gave rise to its name. The most prevalent types of stones are silica, calcium oxalate, urate, struvite (magnesium ammonium phosphate), and cystine.[6] Patients with increased oxaluria also ingest large amounts of oxalate, which is present in nuts and vegetables. An uncommon autosomal recessive disorder, primary hyperoxaluria

typically manifests in childhood. Under a microscope, calcium oxalate stones are visible . Dumbbells can also be made out of them. The most frequent cause of kidney stones in humans is calcium oxalate crystals in the urine, which can also be a potentially harmful consequence of ethylene glycol intoxication [7].

Three mineral species—weddelite, a dihydrate; whewellite, a monohydrate known from some coal seams; and kaoxide, an extremely uncommon trihydrate—are found in nature that include hydrated versions of the chemical.

Patients with increased oxaluria also ingest large amounts of oxalate, which is present in nuts and vegetables. An uncommon autosomal recessive disorder, primary hyperoxaluria typically manifests in childhood. Under a microscope, calcium oxalate stones are visible [8]. Dumbbells can also be made out of them. The most frequent cause of kidney stones in humans is calcium oxalate crystals in the urine, which can also be a potentially harmful consequence of ethylene glycol intoxication. Three naturally occurring mineral types contain hydrated versions of the chemical: weddelite, a dihydrate; whewellite, a monohydrate that is known from some coal seams; and kaoxide, an extremely uncommon trihydrate [9].

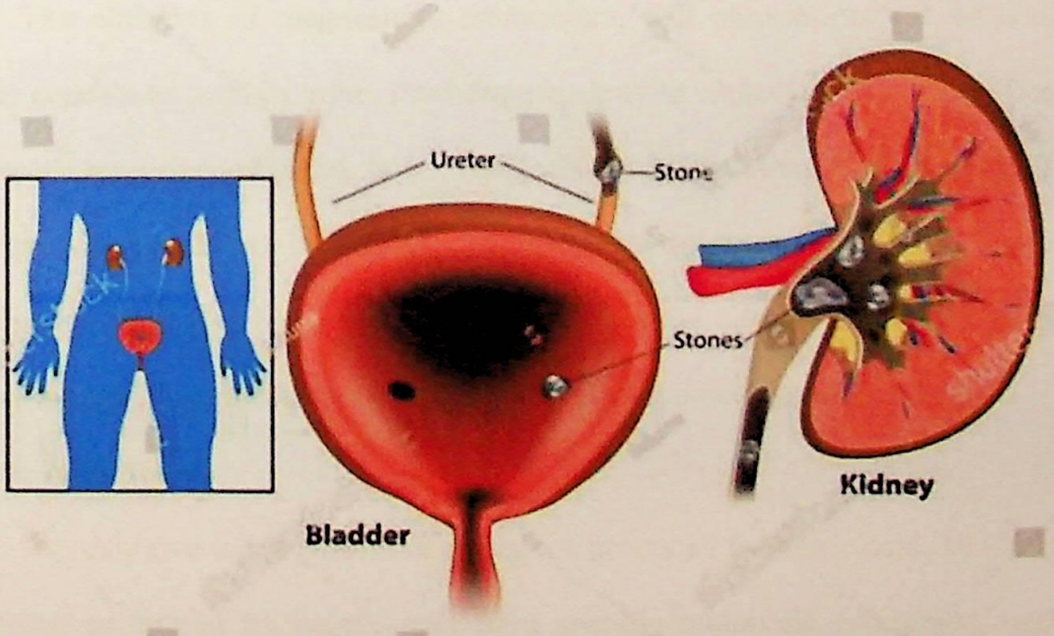


Figure 1.1 Kidney Structure

1.4 Struvite Stone

The material known as struvite, or ammonium magnesium phosphate, makes up roughly 10-15% of urinary stones. Often called "infection stones, urease or triple phosphate stones," struvite stones are most frequently caused by an infection caused by bacteria that degrade urease. These organisms convert urea into ammonia and carbon dioxide by means of the urease enzyme. As a result, the urine becomes alkaline, which encourages the formation of struvite stones.

Individuals with ileal urinary tract syndrome, vesicoureteral reflux, obstructive uropathy, spinal cord injuries, and other forms of neurogenic bladder are frequently affected by these stones and more than average risk of UTIs. Moreover, individuals with metabolic diseases including gout, hyperparathyroidism, and idiopathic hypercalciuria frequently exhibit them [10].

Large calcareous hornstones, which can be caused by infectious stones, can grow quickly and necessitate invasive surgery like percutaneous nephrolithotomy for effective therapy. Under a microscope, struvite stone (triphosphate/magnesium ammonium phosphate) has a coffin lid and a distinct shape.

The elements of magnesium, ammonium, and phosphorus are what allow struvite crystals to form in urine. Furthermore, struvite crystallization is significantly influenced by urine pH and how it affects trivalent ionic phosphate (PO4-3) concentration. Urine pH rises cause H3PO4, H2PO4-1, and HPO4-2 to quickly deprotonate, or remove hydrogen ions, which raises the concentration of PO4-3, the primary ingredient and force behind the creation of struvite crystals [11].

1.5 Uric Acid Stones

Roughly five to ten percent of stones are produced from uric acid. 18 Uric acid stones can be caused by people who have specific metabolic conditions, such as

obesity. Furthermore, patients without hyperuricemia (high uric acid in the serum) or with hyperuricosuria (excessive uric acid in the urine) may also exhibit them. They may also be associated with acid/base metabolic issues, where an extremely acidic (low pH) urine causes uric acid crystals to precipitate. Radiating stone and uric acid crystals in fresh urine samples support the diagnosis of uric acid Urolithiasis [12].

1.6 Cystine Stone

Cysteine kidney stones are caused by a hereditary (genetic) transport defect of the amino acid (protein building block) called cystine, which is referred to as cystinuria. Cysteine stones and an excess of cystine in the urine are the outcomes. Cysteinuria is the most common defect in amino acid transport.

Among the naturally occurring amino acids, cystine is the least soluble, although not being the only one that is over secreted in cystinuria.Cysts typically settle in the urine and develop into urinary tract stones. Urine contains little stones that are eliminated [13].

However, medium-sized stones travel from the kidney to the ureter and deposit there, further restricting the flow of urine , while large stones remain in the kidney (kidney stones), which hinders the flow of urine. Pressure builds in the kidneys and urethra when the urine tract is obstructed. causes the kidney to get compressed and the ureter to dilate. Urine that is blocked also becomes stagnant, which invites the development of recurrent UTIs. Kidney disease is brought on by pressure on the kidneys and urinary tract infections. Kidney dialysis or an organ transplant may be necessary if the damage worsens and leads to kidney failure.

All the indications and symptoms of cystinuria, such as the following: Hematuria, or blood in the urine; Flank discomfort, or side pain brought on by kidney pain; Renal colic, or severe, cramping pain brought on by urinary tract stones;

Obstructive uropathy: obstruction-related urinary tract illness • Urinary tract infections [14].

1.7 Calcium stone

One of the ingredients of calcium oxalate, the usual kidney stone in humans, is calcium. High dietary calcium intake, in contrast to supplementary calcium, does not cause kidney stones and may even prevent them from forming. This could have something to do with how calcium helps the gastrointestinal system bind oxalate that has been consumed. The amount of oxalate absorbed into the bloodstream rises with a decrease in calcium intake; The kidneys subsequently excrete more of this oxalate in the urine. It lowers the chance of stone development since magnesium, like citrates, also inhibits the formation of crystals in the urine [15].

However, interest in allopathic medications has grown, such as beta-carotene and statins combined for the therapy of urolithiasis. In this analysis, we employ a rodent model to determine the anti-urolytic effect of statin and beta-carotene combination. We also explain its usage in urolithiasis and investigate potential anti-calcium oxalate crystallization, antioxidant, and kidney cell properties. Its anti-urolytic activity is likely attributed to its diuretic and protecting properties [16].

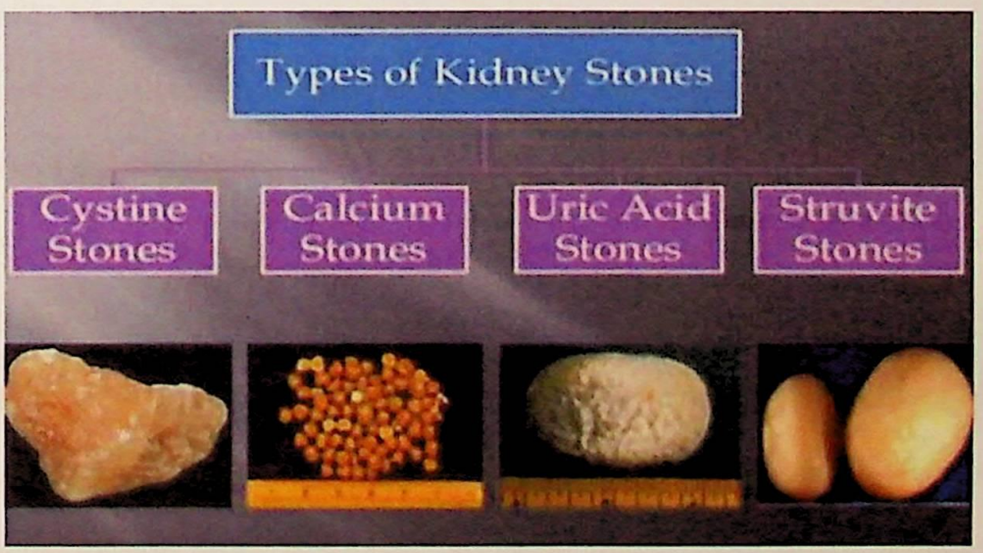


Figure 1. 2 Types of Kidney Stones

Cytochrome P450 (CYP) 3A4 is the primary metabolizer of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is inhibited by atorvastatin. According to a recent study, the CYP3A5 polymorphism influences how well statins cut cholesterol. Thus, we looked at the possibility that CYP3A5 influences atorvastatin metabolism. Human liver microsomes and recombinant human CYP3A enzymes produced the atorvastatin metabolites para- and orthohydroxyatorvastatin; the kinetic behavior of the enzymes suggested substrate inhibition [17].(Mansour Almazroui et al. 2012)

When compared to the CYP3A5 CLint rate, the internal clearance (CLint) of para- and ortho-hydroxyatorvastatin by CYP3A4 was 2.4 and 5.0 times, respectively. This indicates that CYP3A4 is the primary P450 isoform in charge of atorvastatin metabolism. The findings says that CYP3A5 genetic variation may not have a significant role in the inter individual variability observed in atorvastatin distribution and pharmacodynamics in humans, as CYP3A4 is the primary enzyme responsible for metabolizing atorvastatin, not CYP3A5. The potential of statin therapy to avert diabetic kidney damage is unknown. Therefore, it is unknown if the rising usage of statin medication will have a significant effect on the frequency of renal problems in the future [18].

The impact of statins on urine albumin excretion or glomerular filtration rate (GFR) in diabetes has not been thoroughly examined in many large-scale research to date.1. There was conflicting evidence in a recent studies on the impact of statins on these outcomes in the populations with and without diabetes. and only had information from 548 patients.[12] As such, the primary goal is to assess how atorvastatin affects estimated GFR (GFR) and albuminuria. in the Collaborative Atorvastatin in Diabetes Study (CARDS), one of the biggest randomized studies (n =

2838) of statin treatment in individuals with diabetes. Just over a fifth of CARDS patients had albuminuria at baseline.

It is now widely known that statin medication prevents cardiovascular disease (CVD) in persons who have diabetes. We previously reported on the CARDS trial, wherein atorvastatin at a dose of 10 mg/day reduced major cardiovascular events by 37%, and that patients with type 2 diabetes who had no prior history of CVD had mean LDL cholesterol (LDL-C) of 46 mg/dl and an average decrease in triglyceride levels of 35 mg/dl.[12]It has been questioned, nevertheless, if statins help diabetic people with reduced renal function avoid cardiovascular disease quite as much.4 There was insufficient evidence in the Die Deutsche Diabetes Dialyse Studie (4-D) trial to support the value of atorvastatin treatment for cardiovascular disease. individuals with last stage kidney disease and diabetes [19].

The dearth of scientific information on this topic was brought to light in the KDOQI (KDOQI) 2007 Clinical Practice Recommendations on Diabetes and Chronic renal disease (CKD)6. He came to the conclusion that there is little data to support the cardiovascular benefits of statins in stages 1-3 of CKD. Patients in stage 1 . only exhibited "moderate" Thus, another goal of this paper is to investigate if atorvastatin's effect on cardiovascular disease in the CARDS trial was influenced by baseline renal state [20].

1.8 HMG-CoA reductase inhibitors

The popular class of drugs known as statins lowers cholesterol by inhibiting the 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase enzyme. They are most frequently used in coronary disease prevention, both primary and secondary. Thus, at least some of the benefits of statin medication may be explained by its impact on nonlipid factors. For instance, one of the primary components of atherosclerosis

that statins inhibit is the inflammatory process of the blood vessel wall. By preventing the activation of monocytes, the production of metalloprotease in the arterial wall, and proinflammatory cytokines like interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1b, statins also slow down the development of atherosclerosis [21]) Additional evidence of statins' anti-inflammatory properties comes from the fact that they decrease the synthesis of cyclooxygenase-2 (COX-2) in cytokine-stimulated cultured vascular smooth muscle cells and in a rabbit model of atherosclerosis. Furthermore, atorvastatin decreased joint hypernociception in a number of inflammatory disease models, including the collagen and complete Freund's adjuvant (CFA)-induced arthritis and the CFA arthritis model of experimental encephalomyelitis. Statins also prevent inflammatory processes. Statins help prevent cardiovascular disease because, in addition to their effects on cholesterol synthesis, they also raise the expression and activity of endothelial nitric oxide synthase (eNOS) [22, 23]).

Chronic kidney disease is frequently linked to notable changes in fat metabolism (CKD). These include decreased levels of lipoprotein (Lp[a]), cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (VLDL), and triglycerides, as well as increasing levels of low-density lipoprotein (LDL).Three or four more irregularities. comprises an increase in Apo B, a decrease in HDL2 cholesterol, and an increase in the ratio of Apo C to Apo C-II.4-6 Individuals with proteinuria, particularly those with nephrotic syndrome, have more severe dyslipidemia [24].

Compared to the general population, individuals with chronic kidney disease (CKD) have higher rates of cardiovascular morbidity and mortality due to lipid abnormalities.8–10 Additionally, there is evidence to show that dyslipidemia may accelerate the development of kidney disease. For both type 1 and type 2 diabetics, hypercholesterolemia raises the risk of declining renal function [15].High levels of

Apo B are highly linked with the rate at which renal function diminishes in patients with chronic kidney disease (CKD), regardless of the presence of diabetes. -Diabetic patients with hypertriglyceridemia and hypercholesterolemia progressed through renal disease more faster than those with normal lipid levels, regardless of blood pressure [25]. A faster decline in GFR is also reliably indicated by low serum HDL in patients with nondiabetic renal impairment.16 It has been shown that statin therapy shortens the progression of kidney disease in animals [14]. Only a limited number of studies and patient assessments have examined the impact of statins on the advancement of renal disease in humans.19, 20 In patients with CKD and proteinuria from idiopathic glomerulopathy, the purpose of this study was to ascertain the impact of atorvastatin, a 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, on the course of renal disease [26].

1.9 Caroteniod

The most prevalent carotenoid in human diets, β-carotene (BC), is virtually solely found in the (all-E) isomer. BC (Z) isomer concentrations are high in processed foods and animal tissues. The varied propensities of different BC isomers to create retinas, prevent cancer, and prevent heart disease are reviewed. Carotenals and other breakdown products are produced when BC is cleaved eccentrically. A large class of naturally occurring pigments that dissolve fat called carotenoids. Eighty to ninety percent of carotenoid intake in affluent nations comes from fruits and vegetables. Roughly one-third of the over 700 naturally occurring carotenoids that have been identified to far. 50 are present in human diet, which the body absorbs and processes [27]. But only six of them make up more than 95% of all blood carotenoids: lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and β-carotene. Naturally occurring orange-colored carbon-hydrogen carotenoid, β-carotene (BC), is abundant in dark green leafy

crops and yellow-orange fruits and vegetables [28]. Furthermore, the carotenoid is the one that is most frequently present in diet .

Plants naturally produce pigments called carotenoids, which give a variety of fruits and vegetables their vibrant hues. The foods we eat include dozens of different types of carotenoids, most of which have antioxidant qualities. Since b-carotene is the carotenoid most often found in fruits and vegetables across most nations, it has been the subject of the most research. But in the US, tomato-derived lycopene consumption is roughly equal to that of b-carotene. Studies have been done on the possibility that antioxidants, such carotenoids, can prevent chronic diseases. B-carotene and other carotenoids have antioxidant properties both in vitro and in animal models. When carotenoids are combined with other antioxidants, such vitamin E, their ability to combat free radicals is boosted. By utilizing animal models, carotenoid [29].

Natural colors called carotenoids are produced by microbes and plants, not by animals. The categories for carotenoids are as follows: Calotenes are hydrocarbons that are classified as carotenoid and have unique end groups. There are two acyclic end groups in lycopenes. There are two cyclohexene-type end groups in b-carotene. Xanthophylls are oxygenated carotenoids. [30].



Figure 1. 3 Source of Beta Carotene

1.10 Chronic Renal Disease

In the US, chronic renal disease has a significant financial and health-related cost. A recent report from the Disease Control states that one in three persons in America is at high risk of getting kidney disease, and 26 million adults in the country now have kidney disease.1. Among other things, the two main causes of chronic kidney disease are diabetes and hypertension. Age, obesity, smoking, and cardiovascular disease are risk factors.2. Numerous studies have looked at possibly modifiable factors that could affect the risk of CKD before the onset of major clinical risk factors, however the overwhelming body of evidence supports minimizing or postponing the risk of CKD with optimum treatment of these disorders [31]. Based on the limited evidence that is currently available, a diet that is high in fruits, vegetables, whole grains, low-fat dairy, nuts and seeds, legumes, and low in sodium, saturated fat, cholesterol, sugar-sweetened beverages, and red wine is recommended.

Meat may be linked, in individuals spanning the risk spectrum, to a decreased risk of chronic kidney disease. avoided looking at objectively observed dietary indicators in connection to kidney health and instead concentrated on eating habits or particular nutrients obtained from self-reported dietary intake. Dietary questionnaires are a useful tool for evaluating dietary patterns in large cohort studies, but the information they provide is restricted in terms of inferences regarding the association between the risk of disease and the individual bioavailability of dietary components [32].

1.11 ANTIOXIDANTS

Humans can now convert fats, proteins, and carbs into energy because they can use oxygen, but this skill has a cost. Extremely reactive elements like oxygen can combine to form potentially dangerous compounds known as "free radicals." The body's healthy cells can suffer damage from free radicals that results in a loss of

structure and functionality. Free radical-induced cell damage is thought to have a major impact on the aging process and degenerative aging disorders such as cancer, heart disease, cataracts, weakened immune systems, and cognitive impairment. In total, at least 50 distinct diseases have been linked to the pathophysiology of free radicals [33 , 34]

CHAPTER: 2

LITERATURE REVIEW

2.LITERATURE REVIEW

2.1 Urolithiasis Formation

In humans, urolithiasis is a prevalent condition. Despite the fact that urolithiasis is constituted of various minerals, many of the clinical symptoms and diagnostic techniques connected with it are universal across species. To ensure the best possible management, one must be aware of the particular species of minerals. The final identification of a mineral requires removal and chemical analysis. Nonetheless, certain forecasts can be made based on broad epidemiological trends. Urinary stone disorders still have a significant impact on daily urological activities [35]

.According to reports, the typical lifetime risk of stone formation is between 5 and 10%. There is a discernible gender gap, with a peak in occurrence occurring in decades of life. Patients with stone illness often receive treatment for recurrent stone development. The most prevalent kind of stones are those that contain calcium, specifically calcium oxalate monohydrate, calcium oxalate dihydrate, and basic calcium phosphate, which account for 75–90% of cases. Struvite, or magnesium ammonium phosphate, comes in second at 10% and uric acid, at 3%. 10% and 0.5–1% cystine. Generally speaking, calcium oxalate or magnesium ammonium phosphate types 2 and 3 are the most prevalent types of stones [36 , 37].

Over the decades, numerous treatments have been employed to address urinary stones. The majority of treatments in conventional medical systems were determined to be effective and came from plants, yet systematic research has not been able to definitively determine why these medicines were used[38]. Urinary tract stones affect 5–10% of the population in wealthier countries, although they are extremely rare in other countries like Greenland and Japan. Urolithiasis is considered

a severe societal concern due to its high incidence and recurrence rate. In the current day, urolithiasis has to be viewed as a "disease in evolution" because of changes in epidemiology, how those who are "at risk" of stone sickness are treated and prevented, and advances in diagnostic technologies [39].

Numerous social, economic, and cultural elements that are discussed below have caused changes in several aspects of stone disease in recent years. Clinical symptoms have changed as a result of the rising incidence of tiny urinary stones, including persistent pain, hydronephrosis, and frequent episodes of renal-ureteric colic. Similar to this, the utilization of minimally invasive surgical procedures in the treatment of tiny stones has drastically changed due to the existence of leftover fragments following extracorporeal shock wave lithotripsy. While it is extremely uncommon in Greenland or Japan, 5–10% of people in developed nations suffer with urinary tract stones [40 , 41]. This may be caused by a number of factors, including: B. Diet, weather, amount of water consumed each day, amount of physical activity, and obesity [42].

The likelihood of acquiring urinary stone disease in healthy persons differs among nations worldwide. Asia seems to have a lower probability of stone production (1–5%) than Europe (5–9%) and the USA (13%). Saudi Arabia has been reported to have the highest risk (20.1%). Urinary stone incidence and prevalence have both increased recently [28]. The annual prevalence of stone illness in Central Europe climbed progressively from 5.9 to 9%, and the hospitalization rate went from 0.049% in 1954 to 0.097% in 1974. Comparing urinary stones to other urological illnesses, the incidence rate was 1.7% in 1994, whereas the prevalence was 17.2% [43].

The geographic location and historical era have an impact on the epidemiology of stone disease. The number and kind of stones have changed as a

result of shifting socioeconomic circumstances, both in terms of where they are found and their physicochemical makeup[28]. Calcium oxalate, which makes up 50–70% of the chemical composition of stones, can be found either pure (30%) or combined with calcium phosphate (40%). Uric acid (10–20%), struvite (ammonium–magnesium phosphate, 5–10%), and cystine (1-2%) are other forms of urinary stones. Rarer stones contain 2,8-dihydroxyadenine, xanthine, and silica [29]. While the percentage of stones formed as a result of infections has drastically dropped from 15% to 4%, the composition of urinary stones has changed in recent years due to a rise in calcium oxalate in the urine (from 65 to 80% [44 , 45].

Both the increased standard of living in industrialized nations and the extensive use of antibiotics may be to blame for this because homozygous cystinuria still affects a relatively tiny fraction of the population, the epidemiology of cystine stones has not changed. Uric acid stone prevalence has also decreased, from 15% to 8%. The early identification of patients' modified metabolic status and the strict diets prescribed to obese individuals may be responsible for the decrease [46]. stone dimensions Over the past 20 years, there has been a discernible rise in the occurrence of tiny ureteral stones. This tendency could be caused by the increasing use of extracorporeal shock wave lithotripsy (ESWL), which is followed by the development in noninvasive diagnostic methods like spiral computed tomography and ultrasonography [47].

2.2 Therapy for Urolithiasis

A major shift in the therapy of the disease has resulted from the increased occurrence of small urinary stones (less than 1 cm), which has changed clinical signs such as hydronephrosis, chronic discomfort, and frequent episodes of renal-ureteric colic [48]. Minimally invasive methods like uretero-litholapaxy or uretrorenoscopy

with intraureteral stone fragmentation are frequently used to remove smaller stones. For medium-sized kidney stones (between 0.5 and 2 cm.

The authors propose that improvements in diagnostic technology and greater dietary consumption of animal proteins are to blame for the decrease in bladder stones [49]. Modern urologists know that the decrease could be attributed to a reduction in malnutrition worldwide as well as a reasonable comprehension of the obstructive phenomena in the urinary system. A complicated model is depicted when examining the mechanisms behind the start and progression of tonic illness, as well as the role of comorbidities and genetic, anatomical, nutritional, and environmental factors [50]. Moreover, a substantial amount of current research has linked dietary factors such daily calorie intake and animal protein consumption to an increased risk of stone formation. Kidney stone prevalence varies with For people under 45, the prevalence is 0.58%, and for people over 65, it is 4.7% [51].

Obesity, hypertension, hyperuricemia, and other comorbidities are likely the cause of this connection. The male to female ratio in Italy is 2:1, which is in line with the likelihood that stones will develop [52]. Urinary stones are comparatively uncommon among Native Americans, Black people in Africa and the Americas, and Israelis who were born there, according to several studies. Ethnicity is discovered to be a secondary risk factor, nevertheless. A polygenic abnormality with incomplete penetrance may be the cause of urinary stones, as evidenced by the 25% of patients with this condition who have a family history of stone illness [53]. Furthermore, it has been amply shown that in nearly 70% of cases, nephrolithiasis is closely linked to familial renal tubular acidosis. Men are impacted more frequently than women, which is most likely [54 , 55].

Kidney stone disease is still a major problem during standard urological therapy. The average lifetime risk of developing stones has been shown to be between 5 and 10%. In this demographic, men predominate over women, and the fourth and fifth decades of life are when the incidence peaks. Treatment for recurrent stone development is frequently provided to patients with stone disease [56]. The most prevalent stones are those that contain calcium, most especially the 75–90% of cases that contain calcium oxalate monohydrate, dihydrate, and basic calcium phosphate. Other common forms of stones include uric acid , cystine , and magnesium ammonium phosphate (Struvite), which is present in 10–15% of cases [57 , 58].

The majority of the time, calcium oxalate or magnesium ammonium phosphate type 2 or 3 stones are the most often occurring types. Urinary stones have been treated with a variety of methods throughout history. With the exception of a few composite herbal drugs and plants, the most of traditional medical systems' treatments were derived from plants and were shown to be effective even if the scientific basis for their usage was not thoroughly established through systematic pharmacological and clinical studies[59 , 60]. The recurrence rate may be decreased by pharmacotherapy. It is important to employ plant products that traditional medical systems claim to have therapeutic benefits [61].

One approach to reducing tissue damage in human disease may be the use of drugs having several protective action mechanisms [62]. Herbal remedies contain a variety of phytoconstituents and work to treat urolithiasis through a variety of methods, including:

- By raising urine volume, pH, and anti-calcifying activity (diuretic activity), it aids in the spontaneous passing of calculi.

- Maintain equilibrium between the urine's crystallization promoter and inhibitor, which influences the crystals' nucleation, aggregation, and growth Control of oxalate metabolism: It increases the antioxidant status of renal tissue, preserves the integrity of the cell membrane, and prevents recurrence (Antioxidant activity); it balances the crystalloid-colloid ratio and enhances renal function, preventing the recurrence of urinary calculi [63]. Shows strong anti-infective activity (antimicrobial activity) against the main causing.
- Shows a noticeable reduction in the discomfort, burning micturition, and haematuria associated with urinary calculi (analgesic and anti-inflammatory action [64].

Triterpenes' protective action against urolithiatic activity: Plant-derived pentacyclic triterpenes that exist naturally have been shown to have a variety of pharmacological effects [65]. Through dilution of urinary stone-forming ingredients and prevention of crystal-induced tissue injury, lupeol has been proven to be effective in lowering the risk of stone formation in animals. The antilithiatic properties of betulin and lupeol, two structurally similar triterpenes, were evaluated [66]. The procedure of implanting a foreign body and then adding ammonium oxalate was modified to cause bladder stones. As a result, the renal tissues experienced increased lipid peroxidation and decreased antioxidant status [67].

When malondialdehyde and subsequent tissue damage were used as a proxy for crystal-induced renal peroxidative alterations, both triterpenes performed just as well .It was discovered that the antioxidant content of the kidney and bladder of rats that develop stones was significantly lower in both enzymatic and non-enzymatic

components [68]. Betulin and lupeol functioned similarly in terms of restoring the thiol status and antioxidant enzymes such as glutathione peroxidase, catalase, and superoxide dismutase. The two substances may function by preventing the aggregation of calcium oxalate crystals and strengthening the body's defensive mechanisms to prevent harmful symptoms brought on by oxalate and the production of free radicals [69 , 70] .

Recurrences of stones are more common in the spring and summer than in the fall and winter, most likely as a result of decreased fluid intake and urine production. High plasma levels of vitamin D as a result of extended sun exposure could be another reason. Geographical factors may indirectly affect temperature, which in turn affects the frequency and prevalence of stone disease [71, 72]. Elevated temperatures can cause an increase in the insensitivity to perspiration, potentially resulting in a higher urine content. Urine crystallization and stone formation are encouraged by this phenomenon. Nutritional practices Recently, the following dietary risk variables were identified: an elevated consumption of animal protein, calcium, and oxalate in addition to a diet heavy in calories [73].

2.3 Calcium associated with stones

It is yet unknown if consuming calcium increases the chance of developing stones. A strong correlation has been found between body mass index and a higher risk of stone development. Investigation of some writers, there is a negative relationship between a patient's urine pH and body mass index [74]. The main benefit of consuming copious amounts of fluids was proven by Borghi et al. in a recent randomized experiment [75]. The development of Randall's plaques and renal colic are two distinct stages in the natural history of urinary stone disease. A stone obstructing the urine tract causes acute renal or ureteral colic. The ureteropelvic

junction, the margin of the pelvis where the ureter meets the bladder, the calyx, and the ureter travel downward when the stone forms [76 , 77].

The size of the stone determines the chance of impaction at these locations; stones less than 4 mm are expelled spontaneously. The stone may enlarge after impact, resulting in renal colic, superior hydronephrosis, and complete urinary tract obstruction. A stone's ability to move freely is caused by a number of variables pertaining to its size, position, and side. A prospective study found that the most important predictor of stone passage is stone size [78].

According to reports, stone size does not predict stone passage via the ureter on its own. Additionally, it was shown that the placement of the stone matters in determining whether or not it can successfully pass spontaneously: only 22% of proximal ureteral stones can flow into the bladder, compared to 71% of distal ureteral stones[79]. Stone size and position are associated to stone passage, as demonstrated by the AUA urinary lithiasis guideline. When treating patients with urinary stones, it is important to accurately identify the elements that affect the stone's travel through the ureter and to take these aspects into account when planning treatment [80].

The identification of specific risk factors for patients classified as "recurrent stone formers" and the significant advancement of non-invasive diagnostic techniques such as spiral computed tomography and abdominal ultrasound have changed the natural history of stone formation, development, and excretion in recent years. The ESWL era, the widespread use of ultrasound, and antibiotics all had a discernible effect on the number of persons with stone formation [81].

Coenzyme 3-Hydroxy-3-methylglutaryl Statins, also referred to as HMG-CoA reductase inhibitors, block the rate-limiting stage of the cholesterol biosynthesis pathway [82]. They are now the mainstay of the medical management of

hypercholesterolemia and have significantly decreased the morbidity and death rate from peripheral artery disease, heart attacks, and adverse cardiac events. It's interesting to note that a plethora of clinical and experimental data indicates that statin benefits extend beyond merely decreasing blood cholesterol levels.7-9 The word "pleiotropic effects," which is used to describe all of the benefits of statins, is believed to be present in statins. statin side effects that are unrelated to cholesterol levels [83].

Apart from the aforementioned pleiotropic effects of statins, positive results of statin therapy have been observed in the disease states of autoimmune illnesses, cancer, sepsis, dementia, osteoporosis, and inflammatory bowel disease in addition to cardiovascular disease (CVD) [85]. Statins seem to be more significant than previously believed when it comes to the therapy of cardiovascular disease[49]. In particular, statin therapy for cardiovascular disease reduces vascular inflammation, enhances endothelial cell function [86, 87].

Treatments for Alzheimer's disease (AD) that alter the condition are being developed. These are expected to delay the onset of the disease or decrease its clinical course. An early and potentially crucial step in the development of Alzheimer's disease (AD) is the accumulation of amyloid-β peptide (Aβ) in the brain. Thus, it is expected that therapies aimed at reducing brain amyloidosis will impact the condition. As a result of the numerous lab studies that demonstrate the connection between cholesterol and the generation and accumulation of Aβ, as well as the epidemiological data that connects cholesterol metabolism to AD, the current consensus is that cholesterol-lowering medications will be helpful in treating AD [88].

The use of statins to treat coronary artery disease has been linked in several epidemiological studies to a decreased prevalence or risk of developing AD [89]. Studies that are both preclinical and clinical must validate these observations. The

former entails assessing statins in one or more animal models of AD to ascertain which aspects of the disease are impacted by statin therapy, how well various statins work in relation to changing these aspects, and the mechanisms by which statins influence AD phenotypes. For the latter, prospective, randomized, placebo-controlled trials are needed to assess the effect of statin therapy on cognitive function and AD biomarker outcomes [90].

The most often prescribed statin in the US, atorvastatin, is the subject of our investigation on its effects. Since the pathophysiology of atherosclerosis involves oxidized low-density lipoprotein, antioxidants have garnered interest as potential preventive treatments for cardiovascular disease.1, 2 Antioxidant supplementation has not been demonstrated to protect individuals against cardiovascular events, despite promising experimental data [91].Supplementing with vitamin E (alpha-tocopherol) has been linked in observational studies to a lower risk of later coronary events.3,4 Large-scale randomized studies on beta-carotene supplements have either not demonstrated a decrease in cardiovascular events [92].

Illnesses, or possibly a rise in the frequency of deaths from cardiovascular disease. Vitamin E treatment dramatically decreased the incidence of non-fatal myocardial infarction in patients with coronary disease in a placebo-controlled experiment.8 The AlphaTocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study)6 has yielded preliminary results. Specifically, subjects taking beta-carotene supplements had a higher death rate than those taking vitamin E supplements; however, subjects taking vitamin E supplements had a lower death rate from coronary heart disease. The effects of beta-carotene and vitamin E on the risk of primary nonfatal myocardial infarction and fatal coronary artery disease are the main topics of this article [93].

2.4 Chronic Kidney Disease

Chronic kidney disorders are frequently linked to significant abnormalities in fat metabolism (CKD). Reduced levels of triglycerides, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and lipoprotein(a) (Lp[a]) are a few of these.1-3 A higher ratio of apo C to apo C-II, a decrease in HDL2 cholesterol, and a rise in apolipoprotein B (Apo B) are among the other anomalies.4-6 Individuals with proteinuria, particularly those with nephrotic syndrome, have more severe dyslipidemia.3,7 These are lipid-related abnormalities. The fact that cardiovascular morbidity and death rates were higher in CKD patients than in the general population is one of the contributing causes.8–10 Moreover, there is data indicating that dyslipidemia could accelerate the development of kidney disease [94 , 95].

Hypercholesterolemia increases the risk of renal failure in both type 1 and type 2 diabetics.11,12 There is a strong association between high apo-B levels and the pace of decline in renal function in both diabetes and non-diabetic people. individuals with diabetes who have chronic renal dysfunction.Thirteen In those who do not have diabetes individuals with hypertriglyceridemia and hypercholesterolemia progressed more quickly toward renal impairment than individuals with normal lipid levels, regardless of blood pressure.15, 14, A faster decline in GFR is also independently predicted by low blood HDL in patients with non-diabetic renal impairment.16 Studies conducted on laboratory animals have shown that treatment [96].

2.5 Pharmacokinetics of Atorvastatin

Atorvastatin pharmacokinetics may be influenced by the hepatic uptake transporters OATP1B1 and maybe others in ways that are not yet understood. Here, we investigate the effects of rifampin, a model hepatic transporter inhibitor, on the atorvastatin metabolite kinetics in humans. Using a randomized crossover design, this

study investigated the effects of a single rifampin dosage on the atorvastatin kinetics in eleven healthy individuals. On study days 1 and 8, atorvastatin 40 mg dosages were administered to each patient one week apart. All participants received a 30-minute intravenous rifampin infusion of 600 mg on Study Days 1 or 8. Atorvastatin and its metabolites were detected in plasma at levels above detection limits up to 24 hours after dosing. The entire area under the plasma concentration-time curve (AUC) of atorvastatin acid rose significantly by 6.872.4 times with rifampin and by 3.972.4 times with 2- and 4-hydroxy-atorvastatin acid, respectively. The 2-hydroxy- and 4-hydroxy-atorvastatin lactone derivatives of atorvastatin likewise have significantly increased AUC values, albeit not as much. It was shown that the plasma concentrations of atorvastatin and its acid and lactone metabolites were dramatically elevated after an intravenous administration of rifampin. The majority hepatic absorption pathways for atorvastatin and its active metabolites are confirmed by data to involve OATP1B transporters [97].

These long-lived active metabolites are primarily responsible for the circulatory inhibitory effect of 3-hydroxy-3-methylglutaryl coenzyme. The enzyme that limits the liver's ability to produce cholesterol from scratch is called reducase. If there is a persistent reduction in the liver's capacity to absorb atorvastatin and generate its active metabolites, then long-term atorvastatin therapy may cause adverse consequences such as rhabdomyolysis or myopathy. Moreover, this can raise the medication's systemic exposure and decrease its therapeutic efficacy.

Therefore It is necessary to further understand the processes behind the drug interactions that could cause these side effects. Atorvastatin is an effective way to lower low-density lipoprotein cholesterol (LDL-C) levels; however, research on long-term survival has not been done to demonstrate that this drug also reduces

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cardiovascular morbidity and death in high-risk individuals [52]. Some have proposed that the effects of simvastatin and pravastatin in primary (WOSCOPS)1 or secondary (4S, CARE, LIPID)2-4 prevention of coronary artery disease (CHD) are due to the actions of the pharmaceutical class. Conversely, evidence-based medicine demands documentation for every drug in the class. Most of the seminal statin survival studies for secondary CHD prevention2-4 were published by the time atorvastatin became generally available.When compared to the placebo, these demonstrated a definite advantage in terms of morbidity and death [98].

The findings of the Heart Protection Study, which show that no cholesterol level is too low to help the cardiovascular system in those at risk for cardiovascular disease, are probably going to offer more encouragement and possibly even permit patients with "favorable" lipid profiles to continue receiving treatment. A threefold rise in statin use is predicted, with 36 million people expected to use them. Moreover, media reports cite experts who claim statins are safe and effective enough to be added to the water supply. Given these diseases and the dramatic increase in the number of people who can now take them, it is becoming increasingly critical to understand the whole spectrum of cardiac and noncardiac adverse effects of statins [99].

2.6 Beta Carotene Activity

The carotenoid component most commonly found in human tissues, including blood, is β-carotene, which is also widely present in the human diet. Its strong bioactivity makes it a popular choice in medicine as well. Of all the many roles that β-carotene plays in the human body, the most critical one is linked to the production of provitamin A; it also affects proper growth, development of the embryo, and vision. It possesses antioxidant and anti-cancer qualities, and it is thought to suppress some gene. Its coloring qualities also contribute to its significance. β-carotene is utilized in

the food industry as an orange-red pigment in a wide range of items, such as tropical fruit-flavored soft beverages that are not heated, cooking oil, cheese, pastries, and ice cream.

It is utilized as a colorant for tablets in the pharmaceutical business and as a bioactive component in creams that shields skin lesions from UV light and oxidation in the cosmetics sector. The development of production methods for β-carotene is impacted by its extensive applicability and strong bioactivity.

High antioxidant status is associated with a lower incidence of degenerative disease, according to epidemiological research. It's possible that diet alone won't provide the ideal consumption of antioxidants; dietary supplements may be necessary, especially for high-risk sections of the population. Consequently, it's critical to confirm that antioxidant supplements are risk-free and have no adverse effects [100]. The toxicity of vitamin E is minimal; double-blind trials using high doses of vitamin E in humans showed no adverse effects and no evidence of mutagenic, teratogenic, or carcinogenic effects. High doses should not be administered to people with bleeding disorders linked to vitamin K, and more research is needed to determine whether taking big amounts of vitamin E over an extended length of time can be hazardous to healthy people. Additionally, beta-carotene has less toxicity.

Third, there is less proof that dietary interventions with antioxidants can reduce the incidence of disease. Since the effectiveness of an intervention in preventing disease cannot be determined until several years after it is implemented, evidence can only be gathered gradually in prospective epidemiological studies on humans. There are numerous ongoing research that must be completed before any definitive findings can be made. Antioxidant ingredients are deliberately added to the diets of a large number of people worldwide. This happens because poor diet and

lifestyle choices may restrict the consumption of these nutrients, and epidemiological studies have shown the public that taking supplements containing these nutrients may, in some cases, lower the risk of degenerative illness.

Vitamin A is a vital vitamin that is required for healthy growth, development, and vision. For most people, therefore, dietary beta-carotene is important. However, what about the millions of people who consume enough animal products like milk and meat to meet their vitamin A needs? Is beta-carotene necessary for those who consume enough preformed vitamin A, or at least highly beneficial .Most scientists and doctors would have answered in the affirmative a few years ago. Some even seemed to think beta-carotene was a miracle medication. According to some descriptions, beta-carotene is an antioxidant that guards against aging, heart disease, cancer, and macular degeneration. It didn't cause any harm. In order to provide healthy, well-fed adults with more beta-carotene, numerous sizable, well-funded trials were established In an attempt to prevent cancer and heart disease, a study was carried out wherein male physicians in good health in the United States of America were given beta-carotene supplements. These physicians were not previously identified as being at-risk for any nutritional deficiencies. Research on carotenoid increase was phenomenal [101].

Measurements of food composition, dietary intakes, and blood and tissue carotenoid concentrations were made possible by the development of useful new techniques. Important new data was gathered regarding the typical food consumption and serum concentrations in healthy, well-fed adults, as well as the processes behind the action of beta-carotene in the immune system, cell-to-cell communication, and antioxidant capacities . There was hope that critical parts of human beta-carotene metabolism could be measured and understood for the first time thanks to improved

animal models and novel stable isotope techniques. But relationships have evolved. Regrettably, it seems that the potential advantages of supplementing healthy, well-nourished people with beta-carotene, or beta-carotene, have been exaggerated.

Most of the studies on supplementing with beta-carotene have yielded poor outcomes. According to two, using beta-carotene supplements may cause harm to smokers who have been smoking for a long time. It is thought that carotenoids other than beta-carotene have a greater bearing on macular degeneration and ocular disorders. According to some researchers, the epidemiological findings that seemed to support beta-carotene have more to do with other carotenoids, phytochemicals, or even lifestyle variations. Research even suggests that preventing vitamin A insufficiency using veggies' beta-carotene may not be very effective.Does this latest setback indicate that beta-carotene is a failed wonder medicine whose time has come and gone, or are we projecting too optimistically? Why is beta-carotene beneficial? How crucial beta-carotene is to overall human health as well as the health of particular communities How much more cash, time, and energy ought to be allocated to studies on beta-carotene? And how and where ought we to use them? And what has actually been achieved over the last several years.

Plant-based diets are rich in phytochemicals, including flavonoids, carotenoids, and other phenolic substances. Research has demonstrated the strong anti-free-radical properties of several phytochemicals, particularly polyphenols, which lower the chance of long-term illnesses like cancer, heart disease, and age-related cognitive decline. The human body produces a range of free radicals during aerobic respiration, such as alkoxyl, superoxide, hydroxyl, hydroperoxyl, and peroxyl radicals. These free radicals are often eliminated by the body's own endogenous

antioxidant enzymes in healthy individuals. On the other hand, the body may combat free radicals by using nutritional antioxidants.

Therefore, Reducing the harmful effects of oxidative stress requires eating a diet high in fruits and vegetables, which are high in antioxidants. Antioxidants include tocopherol, b-carotene, phenolic acids, and anthocyanins are abundant in sweet potatoes. They also include a lot of vitamins, minerals, and dietary fiber. Sweet potatoes' distinct flesh colors—cream, deep yellow, orange, and purple—as well as their antioxidant properties are attributed to carotenoids and phenolic compounds. The two primary phenolic acids found in sweet potatoes grown in the United States were isochlorogenic acid and chlorogenic acid; the levels ranged from 0.14 to 0.51 mg of equivalent chlorogenic acid/g fresh weight of hand-peeled store roots [102].

However, In their analysis, they discovered that the antioxidant activity of an unidentified cultivar of sweet potatoes was lower than that of broccoli and green vegetables. However, it was in the same group as other vegetables like squash, carrots, and potatoes. Sweet potato roots have a broad spectrum of antioxidant properties due to the variation in flesh color, which can be white, yellow, orange, or even purple. To assess the antioxidant properties of naturally occurring substances in food or biological systems, a variety of techniques can be used [68]. Two widely used techniques in measuring antioxidant activity are 2,20-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (ABTS). In these procedures, free radical generators are employed, correspondingly.

Both tactics are based on the same principle: when a molecule is reduced by an antioxidant or a free radical species, the stable free radical's absorption spectrum changes. Unlike DPPH, which often takes many hours to react completely, ABTS reacts rapidly and dissolves in both organic and aqueous solutions. The DPPH test

underestimates antioxidant activity when samples with anthocyanins are used because of color interference. But the ABTS assay does not have this issue, particularly when the absorbance is measured at 734 nm. Nonetheless, a number of researchers have investigated the antioxidant potential of sweet potatoes using the DPPH method. An additional method of determining antioxidant capability is to employ oxygen radical absorbance capacity (ORAC) [103].

This approach suppresses the effects of peroxyl radicals by blocking the oxidation that is triggered by the thermal breakdown of azo-compounds such as 2,20-azobis (2-amidino propane) hydrochloride. The antioxidants stop the fluorescent probe fluorescein from degrading by interacting with peroxyl radicals. The ORAC approach can be easily integrated into high-throughput assay equipment by utilizing biologically relevant free radicals and combining the duration and degree of antioxidant activity into a single data value .The ORAC approach has the benefit of being able to measure total antioxidant activity more accurately because it can assay both lipophilic and hydrophilic antioxidants. To improve the water solubility of lipophilic antioxidants—which are primarily carotenoids and vitamin-related compounds—randomly methylated b-cyclodextrin (RMBC) was employed. The disadvantage of this method is that it usually requires using a specialist tool. investigated the antioxidant activity of a cultivar of sweet potatoes using the hydrophilic and lipophilic ORAC techniques. Several studies have shown correlations between the amounts of phytochemicals in various food items and the antioxidant activity as assessed using different techniques. Sadly, this kind of information about sweet potatoes is scarce. Moreover, a number of reaction processes serve as the foundation for the commonly used methods for determining antioxidant capacity. [69] It is necessary to assess the antioxidant values of sweet potato samples produced

using various techniques in order to choose an appropriate antioxidant capacity assay method that can be quickly implemented in sweet potato breeding initiatives and food processing.

Educating consumers on the antioxidant activity of sweet potatoes may also increase their awareness of amount of health-promoting phytochemicals present in this nutrient-dense produce[69]. The most prevalent carotenoid in human diets is β-carotene (BC), which is nearly exclusively found as (all-E)-isomer. Animal tissues and processed meals both contain sizable concentrations of BC's (Z)-isomers. It is investigated whether different BC isomers can form retina and prevent cancer and heart disease. Carotenals and other degradation products are produced when BC undergoes eccentric cleavage. The non-vitamin A active BC metabolites have been linked to a number of detrimental effects, including elevated CYP activity, impaired mitochondrial function, and increased benzo[a]pyrene carcinogenesis.

The BC isomers showed no ferric reducing activity in the current investigation (FRAP assay). There were no significant differences observed in the αTEAC assay for bleaching ABTS+ or the chemiluminescence assay for scavenging peroxyl radicals (ROO) produced by the heat breakdown of AAPH between the three primary BC isomers (all-E, 9Z, and 13Z). However, due to its limited stability, the (15Z)-isomer was less active[69, 70]. When compared to the parent molecule, the breakdown into β-apo-carotenoids enhanced ROO scavenging and FRAP activity. The order of increasing activity in the αTEAC assay was β-apo-8'-carotenal < β-apo-8'-carotenoic acid ethyl ester < 6'-methyl-β-apo-6'-carotene-6'-one (citranaxanthin), depending on chain length and the type of terminal function found. The outcomes demonstrate that BC's antioxidant effectiveness is sustained throughout time [104].

A broad range of naturally occurring fat-soluble colorants are called carotenoids. In wealthy countries, eighty to ninety percent of the carotenoid intake comes from fruits and vegetables. Approximately fifty of the approximately 700 naturally occurring carotenoids that have been discovered so far are found in human diets and can be absorbed and digested by the body.

But of all the carotenoids found in blood, only six of them—lutein, zeaxanthin, β-cryptoxanthin, lycopene, and β-carotene—account for more than 95 percent. β-carotene (BC), a naturally occurring orange-colored carbon-hydrogen carotenoid, is found in large amounts in dark green, leafy greens and yellow-orange fruits and vegetables. Moreover, the carotenoid is the one that occurs in diets the most frequently. Even though BC isomerizes from trans (E) to cis (Z), unprocessed carotenerich plant sources tend to contain more of the (all-E)-form. Carotenoids may deteriorate and/or isomerize if vegetables with high carotenoid concentrations are stored for an extended period of time or if food is processed.

Though BC (Z)-isomers are also present in human tissues, albeit in minute amounts in circulating human serum, it is believed that these analogues will carry out their designated biological purpose or responsibilities there. Up to 25% of the total amount of BC in human serum was detected in the liver, kidney, adrenal gland, and testes in significant levels of the (9Z)-, (13Z)-, and (15Z)-isomers of BC, although 95% of the total quantity of BC was identified in all-E)-BC. The primary BC isomers' molecular structures in food and human tissues [105].

Beta-carotene, or vitamin A, vitamin C, and vitamin E are among the dietary antioxidants that have been the subject of the most research. The most significant water-soluble antioxidant in extracellular fluids is thought to be vitamin C. It can counteract ROS in the aqueous phase prior to the onset of lipid peroxidation. The

most powerful antioxidant that breaks down chains in the cell membrane to shield membrane fatty acids from lipid peroxidation is vitamin E, a significant lipid-soluble antioxidant. It has been proposed that vitamin C can reproduce vitamin E.

A healthy diet can help avoid many chronic diseases, including cancer, degenerative brain disorders, and cardiovascular diseases (CVD). Food-derived antioxidants, like BC, may be useful in reducing retinal degeneration and cataract development [86]. The incidence of esophageal adenocarcinoma and Barrett's sarcoma is inversely correlated with the consumption of BC, fruits, and veggies, particularly raw fruits and vegetables and dark green, leafy, and cruciferous vegetables, according to numerous epidemiological studies.

esophagus. Furthermore, a diet high in fruits, vegetables, and BC from dietary sources has been shown to protect against lung illness and liver carcinogenesis in a number of studies. Due to its interactions with the chemical composition and cellular membranes (refer to Figure 1), BC might have antioxidant capabilities that are therapeutically important. It is commonly recognized that BC has a significantly greater ability to quench singlet oxygen than α-tocopherol. Furthermore, it has been demonstrated that BC's (Z)-isomers exhibit antioxidant activity in vitro [74]. Conversely, three sizable trials involving BC interventions—the Physician's Health Study (PHS), the β-Carotene and Retinol Efficacy Trial (CARET), and the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBC)—have all demonstrated that synthetic BC has no effect at all on lowering the risk of cardiovascular disease in populations that are well-nourished [106].

BC possesses the highest provitamin A activity among the 50 different carotenoids that can be converted into vitamin A through metabolism. In vertebrates, the conversion of BC into two molecules of retinal is catalyzed by β, βcarotene-15,15'-

monooxygenase (BCMO I). Two derivatives of vitamin A, retinoic acid (RA) and retinol, are produced from the created retinal after further metabolism. The provitamin A activity of (Z)-isomers is substantially lower than that of (all-E)-BC. It is 38%, 53%, and 100%, respectively, for (9Z)-BC, (13Z)-BC, and the (all-E)-form relative bioconversion to retinol.

After incubating BC with liver, kidney, and lung homogenates of rats, ferrets, and monkeys, an eccentric cleavage in healthy animals was also postulated in addition to this central cleavage pathway. The production of β-apo-carotenal derivatives, like β-apo-8'-carotenal (shown in Figure 1), is probably due to stepwise oxidation starting from one end of the polyene chain. The resulting aldehydes were either oxidized by β-oxidation pathways to produce retinoic acid, or they were transformed into β-apo-carotenol, β-apo-carotenoic acids, or their esters. Alternatively, they underwent further cleavage to produce carbonyl compounds with a short chain. The three apo-carotenoids under examination are found in the diets of both humans and animals. Recently, β-apo8'-carotenal and β-apo-8'-carotenoic acid ethyl ester have been found in human plasma from specific fruits.Apart from the enzymatic cleavage of BC that occurs during mammalian metabolism, significant quantities of cleavage products are also generated upon BC's attack by free radicals. For example, intestinal extracts from rats deficient in vitamin A included traces of β-apo-8'-carotenal and 6'-methyl-β-apo-6'-carotene-6'-one (citranaxanthin). The findings suggest that at physiologically relevant quantities of BC and its breakdown products, the cleavage products of BC might be genotoxic. On the other hand, adding BC to primary hepatocytes resulted in a dose-dependent increase in cleavage products and an increase in genotoxicity. BC by alone had no cytotoxic or genotoxic effects [107].

According to the experts, there's a good chance that the carcinogenic effects seen in the β-Carotene and Retinol Efficacy (CARET) Trial and the Alpha-Tocopherol Beta-Carotene Cancer prevention (ATBC) study are caused by BC breakdown products. The physiologically significant characteristics of BC substances, such as controlling gene expression, cellular signal pathways, or the stimulation of detoxifying enzymes, are better understood than their antioxidant ability. Thus, the study's objective was to compare the antioxidant activity of vitamin E (α-tocopherol), another nutrient-relevant molecule, with that of BC isomers and a few non-retinoic metabolites using a variety of in vitro experiments.

CHAPTER: 3

MATERIALS AND METHODS

3 MATERIAL AND METHODS

3.1 Chemicals and Drugs

Atarvastatin was obtained from Horizon Laboratories Pvt LTD Lahore Pakistan, Beta Carotene (vitamin A) was obtained from Prix Pharmaceutica Pvt LTD Lahore Pakistan. Cystone syrup were obtained from Himalaya Herbal Healthcare. Ethyl glycol (MEG Pakistan Chemicals Karachi), Ammonium Chloride (Sultan chemical industries), Calcium oxalate, Calcium Chloride, Sodium Chloride, Sodium Bicarbonate, Sodium Acetate, Hydrogen peroxide were obtained from Ittehad group of Chemicals.



Figure 3.1 Beta Carotene



Figure 3. 2Atorvastatin

3.2 Experimental Animals

This study was carried out on animals after obtaining permission from research ethical committee of RIPHAH International University with an authorized number ruled under the regulation of Institute of Laboratory Animal Resources, Commission on Life Sciences University, National Research Council (1996). Male wister rats were purchased from Veterinary Research Institute (VRI) Lahore. They were 12 weeks old and ranging in weight 140-260 g at the beginning of the experiment. All rats were housed in animal house of Superior University under 12 h light and dark cycles at 22 ± 2 ◦C temperature and 45–55% humidity to acclimatize them. They had free access to food and water ad libitum [108].



Figure 3. 1 Wistar Rats

3.3 Experimental Protocol

Animals were divided into 7 groups having 5 rats in each group (n = 5) for each study model. Group 1 served as a Normal group and treated with only Normal Saline Solution. Group 2 was Served as a Diseased group and was given a Ammonium Chloride and Ethylene glycol water. Group 3 was served as a Standard group and that was treated with standard drug Cystone. Group 4 served as a study group and that was treated with Atorvastatin 1mg / kg only. Group 5 served as a treatment group and that was treated with 3mg / kg. Group 6 served as a treatment group and that was treated with Atarvastatin 1mg and Beta Carotene 10 mg / kg. Group 7 also served as a treatment group and that was treated with Atarvastatin 3 mg and Beta Carotene 30 mg / kg [109].

Group	Group Name	Stone Induction	Drug Dose
Group 1	Normal Control	Normal Saline	N/A
Group 2	Disease Control	EG & AC both for 1 week and EG only for next 14	N/A

		days	
Group 3	Standard Control	EG & AC both for 1 week and EG only for next 14 days	Cystone 1ml/1kg body wt
Group 4	Treatment 1	EG & AC both for 1 week and EG only for next 14 days	1mg/kg body wt
Group 5	Treatment 2	EG & AC both for 1 week and EG only for next 14 days	3mg/kg body wt
Group 6	Treatment 3	EG & AC both for 1 week and EG only for next 14 days	1mg/kg At & 10mg /kg BC body wt
Group 7	Treatment 4	EG & AC both for 1 week and EG only for next 14 days	3mg/kg & 30 mg /kg BC body wt

Table 1. 1 Animal group division and Drug dose for Preventive study model.

3.4 Study on animal model of urolithiasis

Antiurolithic activity of the test material were determined by using animal model of CaOx urolithiasis . Male Wistar rats (weighing 140-260 g) was divided with matched body weights into groups of 4-6 animals each, which will then randomly selected to receive various treatments for preventive study [110].

3.5 Preventive study model

To study the preventive effect of the test material, rats was served as a normal control, received intra-peritoneal (i.p.) injections of normal saline (2.5 ml/ kg), once in 24 hrs. Whereas, untreated group received i.p. injection of normal saline (2.5 ml/kg) along with renal CaOx crystal deposits inducing (lithogenic) treatment for 21 days. The renal CaOx deposits induction was achieved by giving water containing 0.75% (w/v) ethylene glycol (EG) and 1% (w/v) ammonium chloride (AC) for 7 days, following this the water supply was switched to 0.75% EG alone . Treated groups was also received i.p. injection of the drug dissolved in saline once in 24 hrs and simultaneously received crystal deposits inducing treatment similar to the untreated group. Based on the medicinal use of statin and beta carotene was having a doses of 10-30 mg/kg was used in rats .we were also used log doses 10 and 30 mg/kg for its diuretic and antiurolithic effect, optimized in our pilot study. Animal weight and activity was regularly monitored to assess their overall health and those looking lethargic or who have lost excessive weight was also be excluded from the study. Water intake was determined and 24 hrs urine samples were collected immediately before the onset and at the end of total 21 days of treatment. The 3 hrs morning urine for crystalluria study was also collected before collecting 24-hrs urine at the end of 21 days of treatments. The number of the crystals/mm3 was counted under light microscope [111].

3.6 Preparation of 0.75% Ethylene Glycol

Take 75 ml of ethylene glycol in 1000 ml of water and mix well in a standard Manner .

3.7 Prepration of 1% Ammonium Chloride

Take 1ml of Concentrated Ammonium Chloride in 1000 ml of water and mix gently in a standard Manner.

3.8. In vitro experiments:

3.8.1 In vitro crystallization studies

(a) Kinetic study

The effect of the test material on kinetics of calcium oxalate (CaOx) crystallization were determined by the time course measurement of turbidity changes due to the crystal nucleation and aggregation after mixing solutions of calcium (Ca++) and oxalate (Ox). Stock solutions of CaCl2 (8.5 mM) and Na2C2O4 (1.5 mM), containing 200 mM NaCl and 10 mM sodium acetate was adjusted to pH 5.7 the measurement of optical density at 620 nm were used to investigate the event of CaOx crystallization.[96]

(b) Prepration 1 L of Stock solution of CaCl2 (8.5 mM) CaCl2

Component	Amount	Concentration
CalciumChloride (mw: 110.98 g/mol)	58 g	0.5226M

Table 1. 2 Preparation of Stock Solution

Prepare 800 mL of distilled water in a suitable container.

Add 58 g of Calcium Chloride to the solution.

Add distilled water until the volume is 1 L.

Measure the osmolarity using an osmometer: if below 1000 mmol/kg, add more CaCl2; if above 1000, add H2O. (The osmolarity of 1000 mmol/kg is ~0.383 M. Preparation of this calibrated solution is necessary because calcium chloride is hygroscopic, so is not reliably measured by weight. Slight errors in calcium concentration have an outsized effect on synaptic physiology.) solution was stored at 4C. Solution stored under these conditions appears to be stable for several months.

3.8.2 Determination of antioxidant activity

a. Preparation of Homogenizer

Take a 1g of Kidney Sample in 10 ml of Water than homogenize at 600rpm for 10 mnt and than centrifuge the sample when supernant is clear than stop the centrifugation and than perform all the anti-oxidant tests.

b. Catalase Activity

Catalase activity in laboratory was measured by a spectrophotometric procedure measuring peroxide removal. It was a direct assay with pseudo-first order kinetics and is measured by the method of Beers and Sizer. The rate of peroxide removal by catalase is exponential. Catalase was begin to be inactivated by H_2O_2 at levels greater than 0.1 M, when compound I is converted to compound II or III. By the end of the assay, H_2O_2 was consumed and catalase is inactivated. Catalase activity gels were also be used and will be green-blue in color with white broad bands where the enzyme is present .The separation of native protein, the catalase enzyme removes the peroxides from the area of the gel it occupies. Catalase gels (8% gels) was also having a one band that rarely saturates getting larger with increasing catalase activity . The catalase positive control activity is defined in international unit equals (1 unit) as the amount of catalase necessary to decompose 1.0 μM of H_2O_2 per minute at pH 7.0 at 25 °C while H_2O_2 concentration falls from ≈ 10.3 mM to 9.2 mM. The

concentration of H_2O_2 was calculated from absorbance using the following expression: [H_2O_2 mM] = (Absorbance 240 nm × 1000) / 39.4 mol^{-1} cm^{-1}; where 39.4 mol^{-1} cm^{-1} is the molar extinction coefficient for H_2O_2.[97]

c. Superoxid Dismutase

SOD activity was measured by both activity assays and activity gels. In the biochemical method, xanthine-xanthine oxidase was used to generate $O_2^{\bullet-}$ and nitroblue tetrazolium (NBT) reduction will be used as an indicator of $O_2^{\bullet-}$ production. SOD will compete with NBT for $O_2^{\bullet-}$; the percent inhibition of NBT reduction is a measure of the amount of SOD present. Catalase were included to remove H_2O_2 produced by SOD. . The specific activity of both enzymes was reported as units per mg protein, per μg DNA, or per cell. The order of the addition of the reagents into the assay solution is critical. Bovine serum albumin (BSA) was also added to keep the solution from forming precipitates when bathocuproinedisulfonic acid (BCS) was added. BCS (an electron chain-associated free radical production inhibitor) and diethylenetriaminepentaacetic acid (DETAPAC) are both added to inhibit iron-associated redox cycling and free radical production. The original concentration of sample protein were be around 20 μg μl^{-1}. Various amounts of protein are added into tubes until maximum inhibition of NBT reduction (~80%) is obtained. [98]

d. Reduced Glutathione

GPx activity in laboratory according to an established procedure using H2O2 as a substrate However, the assay will be carried out with cumenehydroperoxide or tert-butyl hydroperoxide as the substrate instead of H2O2 to measure total GPx. Tert-butyl and cumenehydroperoxides will both hydroperoxides and both glutathione S-transferases (non-selenium containing peroxidase or selenium independent) and GPx (contains a selenium in the active site) will utilize

hydroperoxides to determine total peroxidase activity. On the other hand, glutathione S-transferases will not detoxify H2O2. Thus, the assay using cumenehydroperoxide or tert-butyl hydroperoxide measures selenium-dependent GPx and activity from glutathione S-transferases (selenium-independent GPx). This assay will be an indirect, coupled assay for glutathione peroxidase. This assay will takes advantage of glutathione disulfide (GSSG) formed by the enzymatic action of GPx and is regenerated by excess glutathione reductase (GR) in the assay[99].

3.8.3 In Vivo Experiments

a. Determination of diuretic activity

The diuretic activity of the test material was studied on Wistar rats of either sex (180-220 g) . Animals were be divided with matched body weight and sex into groups of 6 animals each. Normal and positive control groups was given by normal saline (20 ml/kg) and standard drug: Statin and beta carotene, 10 mg/kg of body weight, respectively. The rest of the groups were given different doses of the test material dissolved in saline. Subsequently, the animals was placed individually in metabolic and diuretic cages. The urine were be collected in graduated cylinders for 6 hrs at 2 hrs intervals. Total urine excreted out was collected and the volume was determine [112].

3.9 Study on animal model of urolithiasis

Antiurolithic activity of the test material were determined by using animal model of CaOx urolithiasis . Male Wistar rats (weighing 180-220 g) were divided with matched body weights into groups of 6-8 animals each, which were randomly selected to receive various treatments for preventive study [113].

3.10 Preventive study model

To study the preventive effect of the test material, rats was serving as a normal control, received intra-peritoneal (i.p.) injections of normal saline (2.5 ml/ kg), once in 24 hrs. Whereas, untreated group received i.p. injection of normal saline (2.5 ml/kg) along with renal CaOx crystal deposits inducing (lithogenic) treatment for 21 days. The renal CaOx deposits induction was achieved by giving water containing 0.75% (w/v) ethylene glycol (EG) and 1% (w/v) ammonium chloride (AC) for 5 days, following this the water supply was switched to 0.75% EG alone . Treated groups will received i.p. injection of the extract dissolved in saline once in 24 hrs and simultaneously received crystal deposits inducing treatment similar to the untreated group. Based on the medicinal use of statin and beta carotene was having doses of 20-60 mg/kg were be used in rats .we were used log doses 10 and 30 mg/kg for its diuretic and antiurolithic effect, optimized in our pilot study. Animal weight and activity were be regularly monitored to assess their overall health and those looking lethargic or who have lost excessive weight were be excluded from the study. Water intake wsa determined and 24 hrs urine samples were be collected immediately before the onset and at the end of total 21 days of treatment. The 3 hrs morning urine for crystalluria study were also collected before collecting 24-hrs urine at the end of 21 days of treatments. The number of the crystals/mm3 were counted under light microscope [114].

3.11 Calcium oxalate crystal deposition in kidney:

Crystal distribution within the kidneys were determined by using the semi quantitaive scoring by the methods used by Vanachayangkul et al. Briefly the crystal deposits in stained sections with visible in a field of 10× magnification were counted

and severity grades was assigned as 0 = < 1 crystals, 1 = ≤ 10, 2 = ≤ 30, 3 = ≤ 50, 4 = ≤ 75 and 5 = > 75 crystals.

3.12 Histology of Kidney

For Histology Kidney sample was stored in 10% Formalin Solution.

3.13 Formation of 10 % Formalin Solution: 10ml formalin in 100 ml of water.

3.14 Biochemical analysis of urine and serum

Urine samples for Ox, Ca++ and Mg++ , citrate, and uric acid (UA) and serum samples for creatinine and BUN contents were determined by using commercially available kits , while urinary inorganic phosphate and protein was determined by using the molybdenum blue reaction [115].

3.15 Data Analysis

The data is expressed in mean ± standard error of mean (SEM) and the median effective concentration (EC50 value) with 95% confidence intervals (CI). All statistical comparisons between the groups was made by means of t- test (comparison between two groups) or One Way Analysis of Variance (ANOVA) with post hoc Dunnett's test. p value less than 0.05 was regarded as significant.

CHAPTER: 4

RESULTS

4.Results

4.1 In Vivo Diuretic activity

Atorvastatin (1mg and 3mg/kg) increase the urine output in wistar rats, whereas Cystone At and Bc significantly decreased the urinary oxalate, also increase the sodium reabsorption and decrease the potassium reabsorption and also gives a no change in the urinary ph. However, these all results of sodium potassium magnesium indicate the significant diuretic effect of drug Atorvastatin and Beta Carotene on rat model

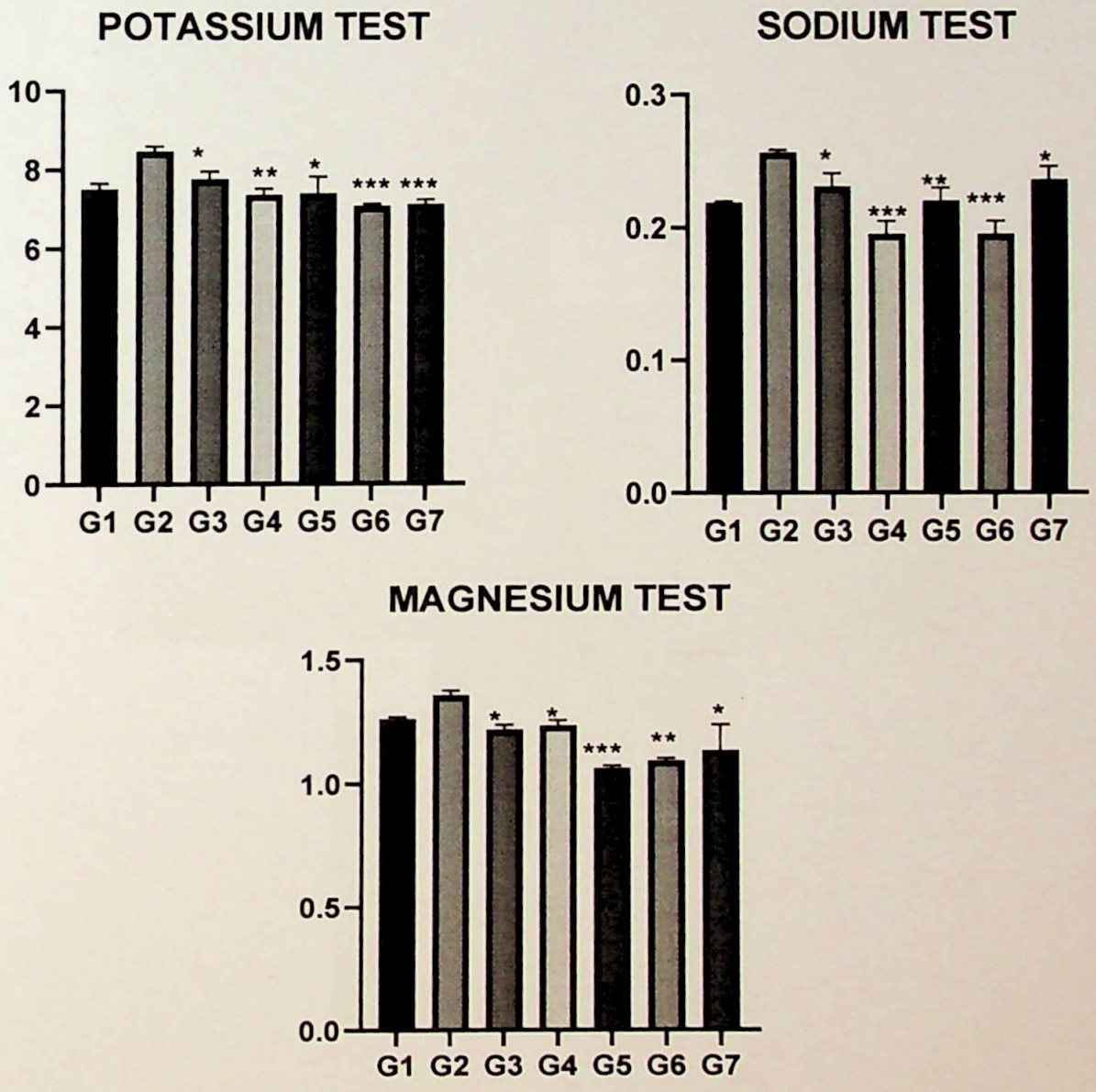


Figure 4.1 Effect of drugs At. Bc and Cystone on N+ and K+

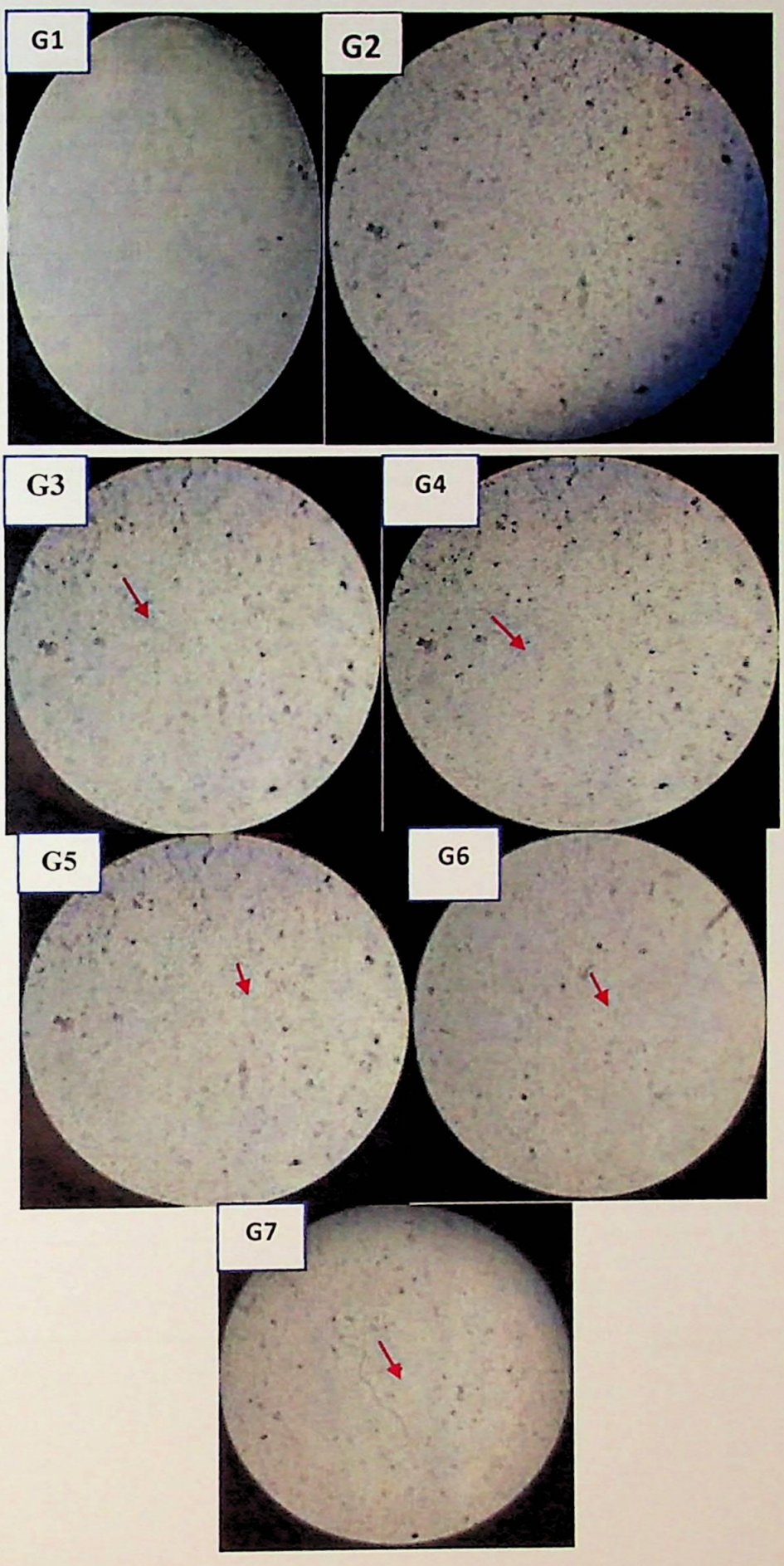


Figure 4. 2 Images of calcium oxalate crystal in 3 hr. morning urine.

CADx

Pet Dated
GRP M6-S1 . 2 (M) / Male

Registration Date
26-Jun-2023 20:29

Owner
- , 03048680185

Reference
Standard

Registration Location
Center for Animal Diagnostics (CADx)

Consultant
-

Patient Number 670001-23-1400013683
Case Number 670018-26-06

Owner Name
M MUZAMIL , Breed Name Other, Species Name Mouse

Department Of Microbiology — Urine Examination

Reporting Time: 27-Jun-2023 13:29

Physical Examination

Colour:	Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.010	Haemoglobin:	+++
pH:	8	Urobilinogen :	Negative
Glucose:	Normal	Bilirubin :	Normal
Ketones:	Negative	Nitrite :	Positive
Proteins:	+	Leukocyte Esterase :	+

Microscopic Examination

Pus Cells:	Nil	Organisms:	Nil
Red Blood Cells:	Numerous	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	
		Uric Acid	Few

Dr. Khizer Hayat
DVM, M.Phil Pathology
Veterinary Pathologist

CADx

Pet Detail
GRP M7-S1 . 2 (M) / Male

Mobile
- , 03048680185

Registration Location
Center for Animal Diagnostics (CADx)

Owner Name :
M MUZAMIL , Breed Name : Other , Species Name : Mouse

Registration Date
26-Jun-2023 20:34

Reference
Standard

Consultant
-

Patient Number : 870001-23-1400013665
Case Number : 870020-26-06

Department Of Microbiology — Urine Examination

Reporting Time: 27-Jun-2023 13:34

Physical Examination

Colour:	Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.030	Haemoglobin:	****
pH:	8	Urobilinogen :	Negative
Glucose:	Normal	Bilirubin :	Normal
Ketones:	Negative	Nitrite :	Negative
Proteins:	+	Leukocyte Esterase :	+

Microscopic Examination

Pus Cells:	Nil	Organisms:	Nil
Red Blood Cells:	Numerous	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	Nil

Dr. Khizer Hayat
DVM, M.Phil Pathology
Veterinary Pathologist

CADx

Pet Dated
GRP3-S.S1 . 2 (M) / Male

Registration Date
26-Jun-2023 19:57

Mobile
- , 03048680185

Reference
Standard

Registration Location
Center for Animal Diagnostics (CADx)

Consultant
-

Patient Number 870001-23-1400013855
Case Number 870012-26-06

Owner Name
M MUZAMIL, Breed Name Other, Species Name Mouse

Department Of Microbiology Urine Examination

Reporting Time: 27-Jun-2023 09:50

Physical Examination

Colour:	Dark Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.010	Haemoglobin:	****
pH:	8	Urobilinogen :	Negative
Glucose:	Negative	Bilirubin :	Negative
Ketones:	Negative	Nitrite :	Negative
Proteins:	*	Leukocyte Esterase :	*

Microscopic Examination

Pus Cells:	Nil	Organisms:	Nil
Red Blood Cells:	Numerous	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil	Dead Sperms:	Numerous
Casts:		Crystals:	
WBC	2-4	Calcuim Oxalate	Few
		Other:	
		Uric Acid	Few

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Veterinary Pathologist

CADx

Pet Detail
G-1 NS-1 . 2 (Y) / Male

Registration Date
25-Jun-2023 21:41

Mobile
- , 03048680185

Reference
Standard

Registration Location
Center for Animal Diagnostics (CADx)

Consultant
-

Patient Number 870001-23-1400013833
Case Number 870022-25-08

Owner Name :
MR. MUZAMMIL, Breed Name : Other, Species Name : Cat

Department Of Microbiology — Urine Examination

Reporting Time: 25-Jun-2023 23:43

Physical Examination

Colour:	Dark Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.030	Haemoglobin:	++
pH:	8	Urobilinogen :	Nil
Glucose:	++	Bilirubin :	Nil
Ketones:	Nil	Nitrite :	Nil
Proteins:	+	Leukocyte Esterase :	Nil

Microscopic Examination

Pus Cells:	Nil	Organisms:	Nil
Red Blood Cells:	Nil	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	
		Other:	Calcium Carbonate
		Triple Phosphate	Few
		Uric Acid	2-3

Dr. Khizer Hayat
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Veterinary Pathologist

CADx

Pet Dated
G-3 DS-1 . 2 (M) / Male

Registration Date
25-Jun-2023 21:22

Mobile
-, 03048660185

Reference
Standard

Registration Location
Center for Animal Diagnostics (CADs)

Consultant
.

Patient Number 670001-23-1400013600
Case Number 670019-25-08

Owner Name
MR. MUZAMMIL Breed Name Other Species Name Mouse

Department Of Microbiology Urine Examination

Reporting Time: 25-Jun-2023 23:41

Physical Examination

Colour:	Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.015	Haemoglobin:	Nil
pH:	8	Urobilinogen :	Nil
Glucose:	Nil	Bilirubin :	Nil
Ketones:	Nil	Nitrite :	Nil
Proteins:	+	Leukocyte Esterase :	+

Microscopic Examination

Pus Cells:	Nil	Organisms:	Nil
Red Blood Cells:	Nil	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	
		Other:	
		Uric Acid	Numerous

Dr. Khizer Hayat
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Veterinary Pathologist

CADx

Pet Detail
GM4 S-1 . 2 (M) / Male

Mobile
-, 03048680185

Registration Location
Center for Animal Diagnostics (CADx)

Registration Date
25-Jun-2023 21:45

Reference
Standard

Consultant
-

Owner Name
MR. MUZAMMIL, Breed Name Other, Species Name Mouse

Patient Number 670001-23-1400013635
Case Number 670024-25-08

Department Of Microbiology Urine Examination

Reporting Time: 25-Jun-2023 23:52

Physical Examination

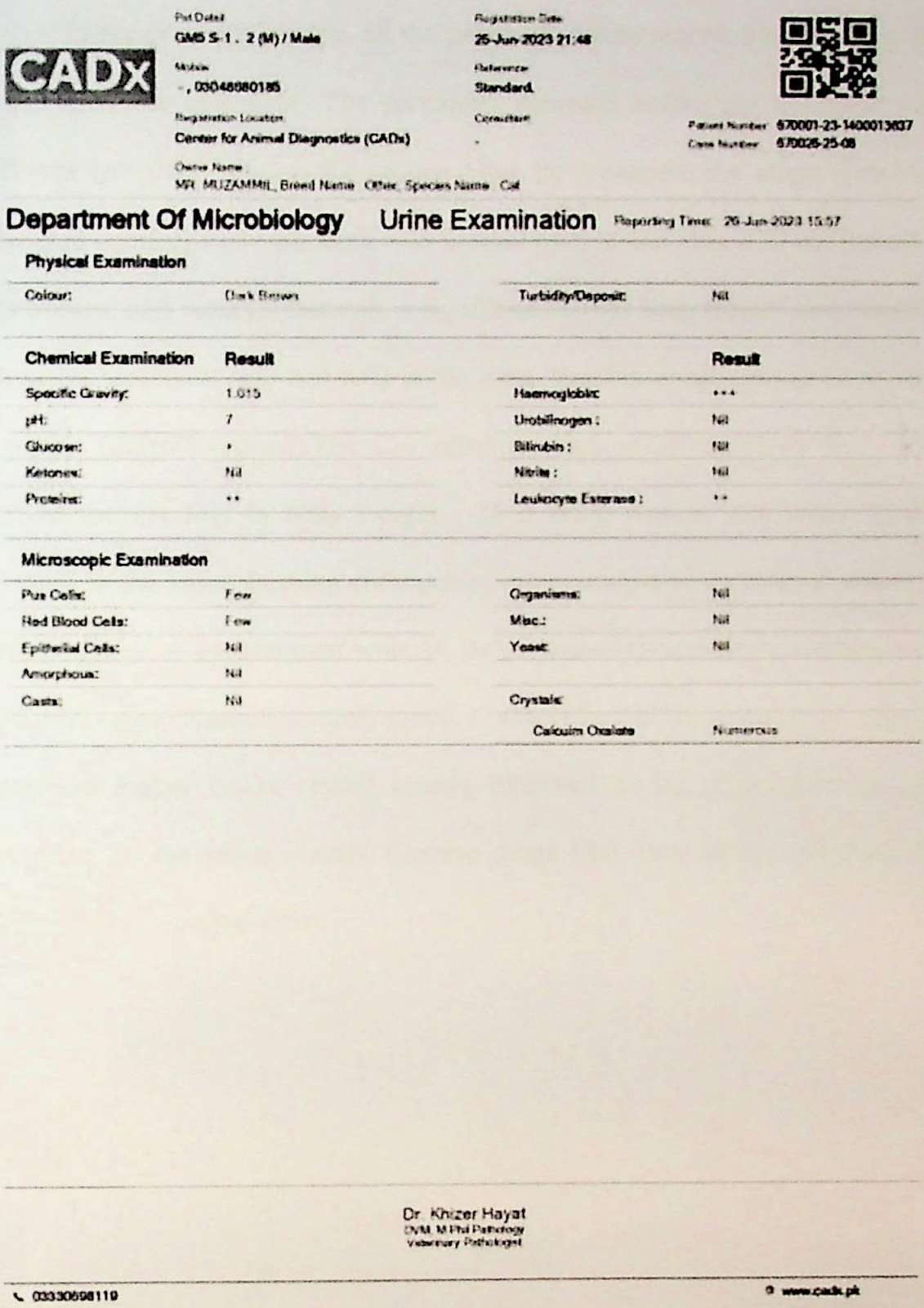
Colour:	Dark Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.020	Haemoglobin:	++
pH:	8	Urobilinogen :	Nil
Glucose:	Nil	Bilirubin :	Nil
Ketones:	Nil	Nitrite :	Nil
Proteins:	+	Leukocyte Esterase :	Nil

Microscopic Examination

Pus Cells:	Few	Organisms:	Nil
Red Blood Cells:	Nil	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	
		Other:	Calcium Carbonate
		Triple Phosphate	Few

Dr. Khizer Hayat
DVM, M.Phil Pathology
Veterinary Pathologist



CADx

Pet Detail
GMS S-1 . 2 (M) / Male

Mobile
- , 03046680185

Registration Location
Center for Animal Diagnostics (CADx)

Owner Name
MR. MUZAMMIL, Breed Name Other, Species Name Cat

Registration Date
25-Jun-2023 21:48

Reference
Standard

Consultant
-

Patient Number 670001-23-1400013837
Case Number 670026-25-08

Department Of Microbiology Urine Examination

Reporting Time: 26-Jun-2023 15:57

Physical Examination

Colour:	Dark Brown	Turbidity/Deposit:	Nil

Chemical Examination	Result		Result
Specific Gravity:	1.015	Haemoglobin:	+++
pH:	7	Urobilinogen :	Nil
Glucose:	+	Bilirubin :	Nil
Ketones:	Nil	Nitrite :	Nil
Proteins:	++	Leukocyte Esterase :	++

Microscopic Examination

Pus Cells:	Few	Organisms:	Nil
Red Blood Cells:	Few	Misc.:	Nil
Epithelial Cells:	Nil	Yeast:	Nil
Amorphous:	Nil		
Casts:	Nil	Crystals:	
		Calcium Oxalate	Numerous

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Figure 4. 3 All Urine Reports

4.2 Effect on Rat model of urolithiasis

In the preventive study, all the parameters were recorded before (day 0) and 3 weeks after the treatment .The parameter recorded before the treatment were not different ($p > 0.05$) among the groups.After the treatment the stone forming group (diseased control) was compered with normal control and also with the treated groups . At the end of 3 weeks treatment , a significant loss in body weight was caused by the lithogenic treatment (EG and AC) in the stone forming group (group II) as compared to saline (normal control).The Co- administration of At.Bc (1,10 & 3 ,30mg/kg) prevent the net loss in body weight , 24 h urine volume and water intake were elevated in the stone forming (lithogenic) group compared to that of saline (normal control) group. A co treatment with At .Bc prevented these urinary changes compared to that of saline (Normal control) group. (Table1) In 3 hr morning urine , significantly more and bigger CaOx crystal mostly observed in the stone forming group as compared `to the saline control whereas drugs like Atarvastatin and Beta carotene reduced urinary crystal count.

4.3 Weight of the Rats

In start , when animals was taking a ammonium chloride and ethylene glycol solution, their body weight was gradually decreased due to ammonium chloride solution effect. When treatment was started decline in the body weight was stopped due to effect of drugs in the body .At the end diseased group animal weight is declined but remaining

Animal Group	Norma-l Control	Diseas-e Control	Standard Control	Treatment Group With Atorvastati-n	Treatment group With Atorvastatin	Treatment group with Atorvastatin & Beta Carotene	Treatment group with Atorvastatin & Beta Carotene
Average Body weight	180-220g	130-170g	170-205g	175-210g	160-205g	140-200g	145-220g

Table 1. 3 Decline in animal body weight

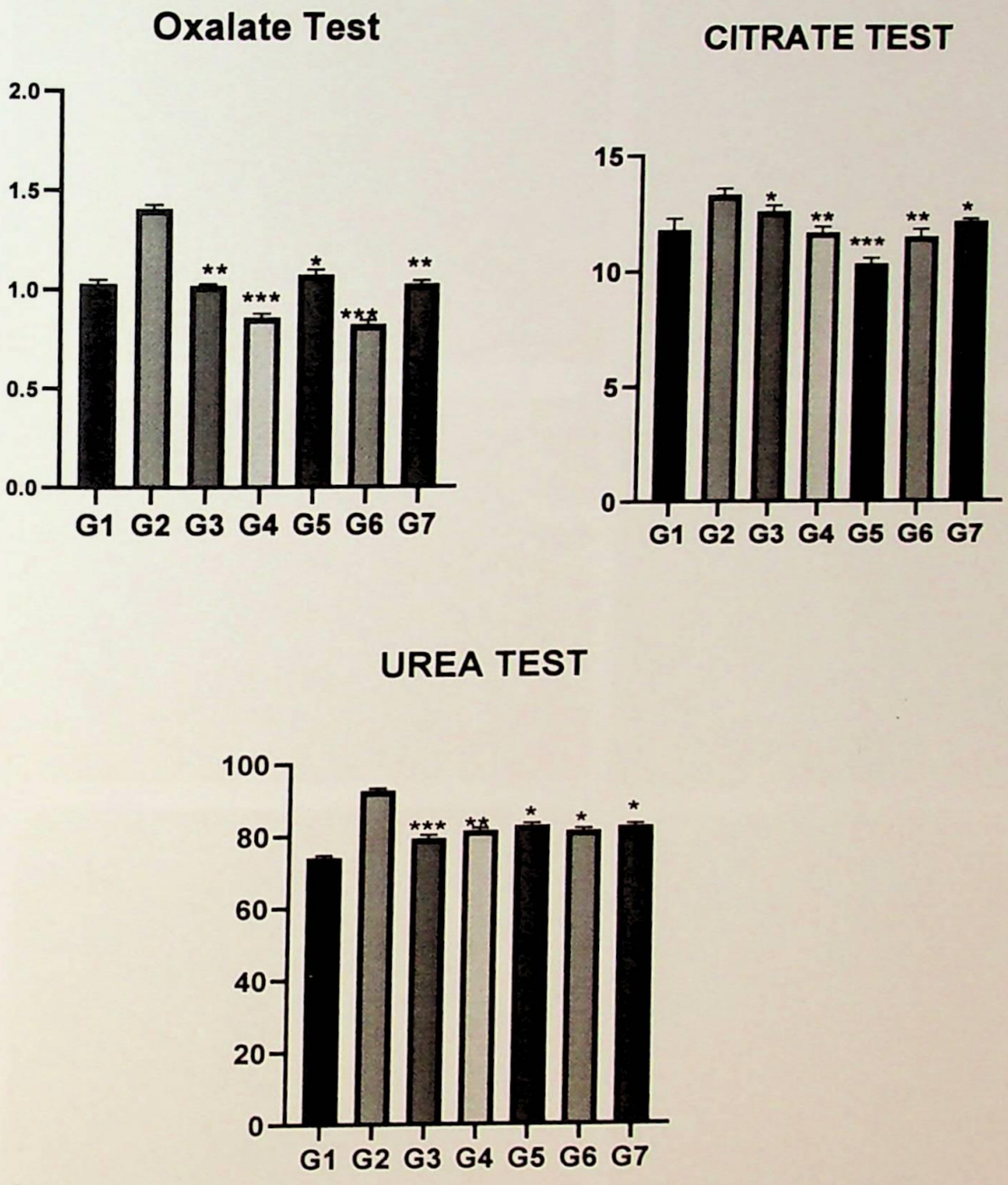


Figure 4. 4 Effect of drugs At. Bc and Cystone on oxalate, citrate and urea

4.4 Histology of Kidney Stone

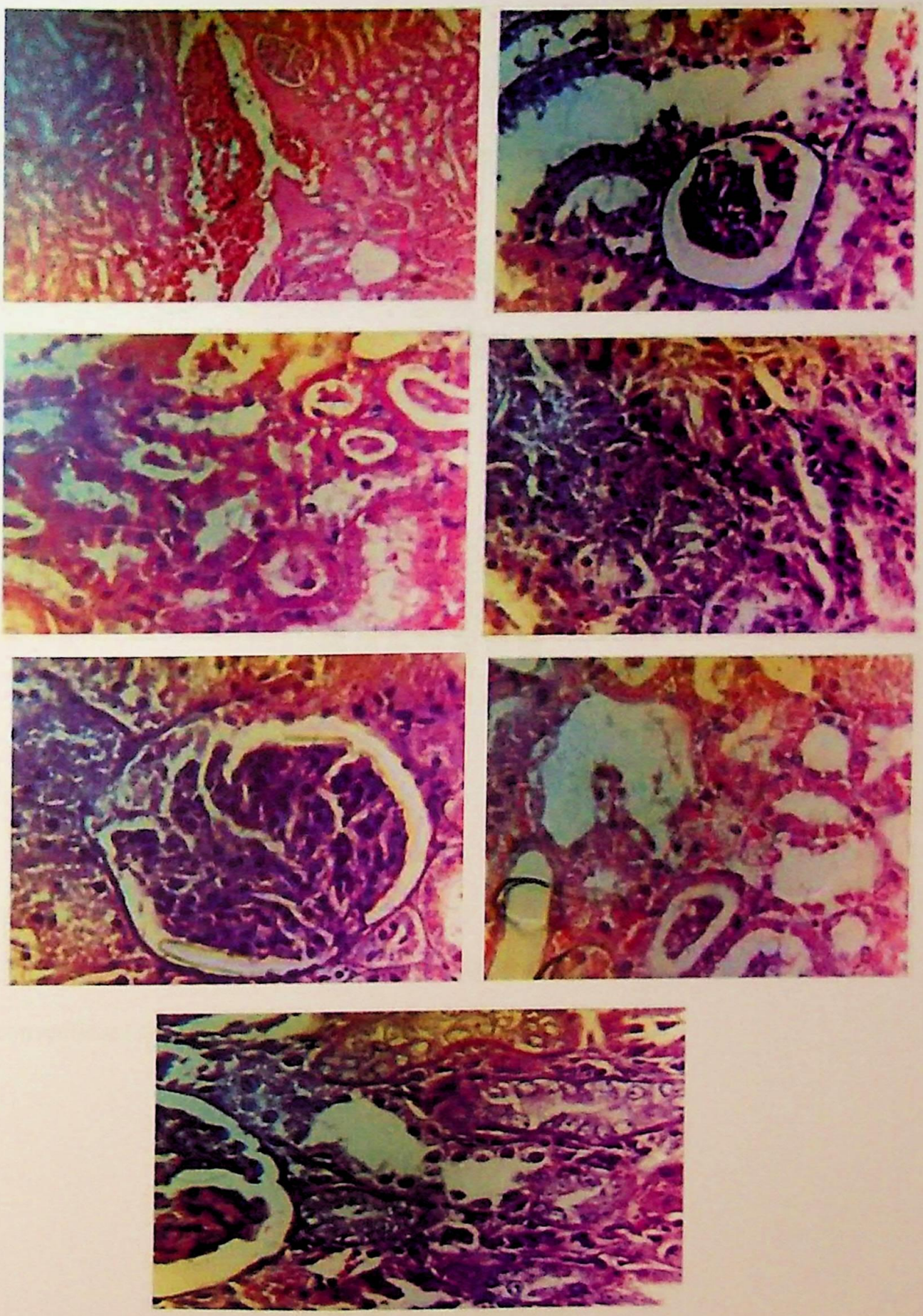
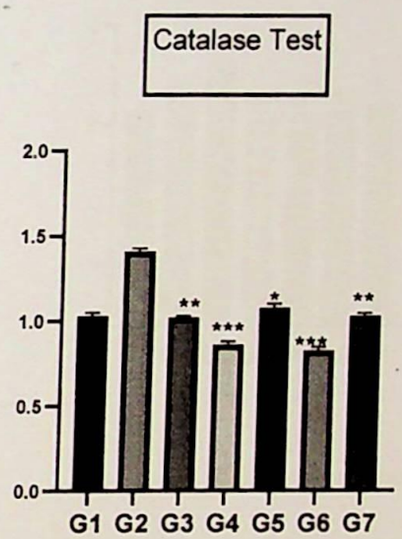


Figure 4. 5 Histology of kidney pictures (100x)

4.5 In Vitro anti-oxidant activity

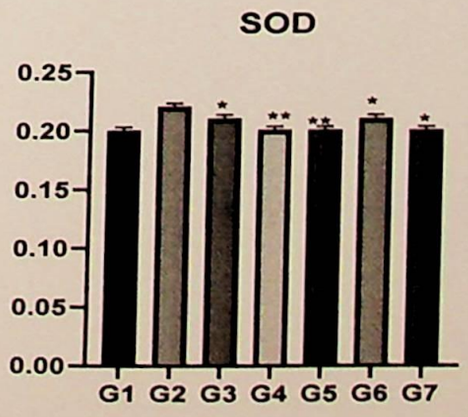
4.5.1 Catalase activity

All animal tissue contains the CAT enzyme, which breaks down hydrogen peroxide and transforms it into water molecule oxygen. The untreated group's level of CAT activity is significantly lower than that of the normal group, but the disease group's level of CAT is significantly higher than that of the standard drug cystone



4.5.2 SOD (Sodium Dismutase)

The kidney tissue homogenate used in this investigation demonstrates that the lithogenic group's SOD level was substantially lower than that of the normal group. In contrast to the disease control group, the SOD level has been dramatically raised by conventional control group cystone and AT.BC.



4.5.3 GSH (Glutathione Peroxides)

The defense mechanism against oxidative stress depends heavily on this antioxidant enzyme (GSH).GSH shields the cell from lipid peroxides and free radicals. The findings indicate that while standard control cystone and AT are present, the lithogenic group's GSH levels are lower than those of the normal group. Comparing BC to the disease control group, the GSH level has increased considerably

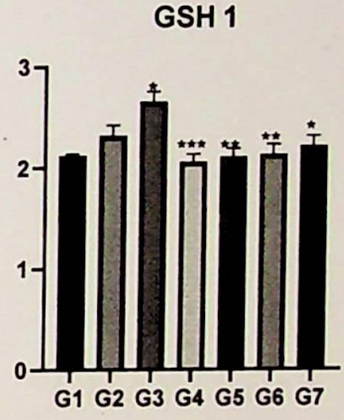


Figure 4.2 Antioxidant Test

CHAPTER: 5

DISCUSSION

5. DISCUSSION

In this Preventive study , Greater crystalluria due to hyperoxaluria induced by EG and AC treatment was observed in the preventive study; total urine protein loss, BUN, and serum creatinine were significantly higher in the control group compared to the disease group (treated with At.Bc) this indicates that increased water intake and urine output could be associated with renal impairment. These findings imply that lithogenic therapy (EG), which blocks At.Bc, raises urine oxalate levels and lowers Ca++ excretion.

Rats who were not given medication showed signs of renal hypertrophy and a generalized buildup of CaOx crystals.. One of the main risk factors for the development of calcium oxide (CaOx) stones is hypercalcuria. It raises the generation of superoxide and hydroxyl free radicals, which eventually causes oxidative stress, cell membrane rupture, and cell death, as several in vivo and in vitro investigations have shown. In the end, these processes lead to CaOx crystal adherence and persistence in renal tubules.

Supersaturation initiation, encompassing crystal nucleation, growth, aggregation, and retention, was carried out to explore other plausible mechanisms that could be implicated in the anti-urolithic properties of At.Bc. While supersaturation is not the only critical phase, it is an essential condition for kidney stone development. Ionic or macromolecular inhibitors of calcium phosphate and oxalate crystallization have been identified by a multitude of investigations. stones found in the urinary tract. Many crystal inhibitors can reduce CaOx supersaturation and halt "crystal nucleation, growth, and aggregation," which reduces the quantity of urine crystallization in those who form stones. Examples of these inhibitors are magnesium oxide and potassium sodium citrate.

Therefore, medications that inhibit the growth and aggregation of crystals may be effective treatment strategies to control the development of recurrent kidney stones. stops CaOx crystals from forming and aggregating at.Bc. These findings suggest that the drug's anti-urolithic impact may be partially attributed to its anti-crystallization action. Thus, oxidative damage brought on by oxalates and crystal deposition can be warded off by antioxidants like beta-carotene and vitamin A.

Studies conducted in vitro and in vivo have demonstrated the detrimental effects of oxalate, calcium oxalate, and hydroxy apatite crystals on kidney cells. Because the generation of ROS causes damage that fosters crystal nucleation, aggregation, retention, and stone formation, it is regarded as a risk factor for kidney crystallization and deposition. Therefore, oxidative damage caused by oxalate and crystal deposition can be prevented by antioxidants such as beta-carotene, vitamin A, and selenium. The measurements were made using free radical scavenging and lipid peroxidation inhibitory activities.

At BC's antioxidant activity Similar to BHT, At. Bc showed the capacity to scavenge free radicals from DPPH and to stop the lipid peroxidation of rat kidney tissue homogenate produced by ferrous ascorbate. The medication atorvastatin lowers cholesterol. It also increases the LDL receptor on the surface of epithelial cells, which inhibits the liver's generation of cholesterol. This involved the combination of beta carotene and atorvastatin for the treatment of urolithiasis. One medication lowers cholesterol, whereas the other Beta-carotene is an antioxidant that directly scavenges reactive oxygen species, preventing cell growth and protein phosphorylation to reduce oxidative stress. When I used both medications together for an anti-urolithic effect, they had an impact on the development of oxalate crystals but were unable to dissolve the oxalate crystals within the body.

They only prevent the creation of stones like those that result from the production of excess lipids and other reactive substances. Both atorvastatin and beta carotene have the ability to lower blood cholesterol and suppress reactive oxygen species, which in turn slows the kidney's process of forming stones.

CHAPTER: 6

CONCLUSION AND FUTURE RESEARCH DIRECTION

CHAPTER: 6

CONCLUSION AND FUTURE RESEARCH DIRECTION

6.1 Conclusion

To support their usage as an antiurolithic agent, the diuretic and antiurolithic properties of the medications atorvastatin and beta-carotene were assessed using both in vitro and in vivo experimental models. At and B.C. significantly enhanced urine output in the in vivo diuretic experiment at doses of 3 and 30 mg/kg, which was comparable to the positive control Cystone (6 ml/kg). Compared to the saline control, the diuretic effect changed less noticeably when 1 and 10 mg/kg of the next dose were administered. Studies have also been conducted on beta carotene's antioxidant and/or synergistic effects. Diuretics decrease the hypersaturation of salts that form stones by increasing urine volume and assisting in the expulsion of crystals.

Our research suggests that At.Bc may be used as a drug to treat urolithiasis because it may have antiurolithic effects against CaOx kidney stone development through a variety of pathways. Before the substance is used in clinical settings, more research may be required to completely comprehend the possible molecular pathways and confirm its safety and efficacy. Future studies should focus on the diverse ways that beta-carotene functions in the body's complex illnesses, which are influenced by these functions. This study revealed that atorvastatin, a lipid-lowering drug, prevents the production of kidney stones such calcium oxalate, and that beta-carotene pro-vitamin A has a strong antioxidant impact that reacts with reactive oxygen species and also data show the antiurolithic action.

When used together, the medications Atorvastatin and Beta Carotene had an impact on kidney stones because the former prevented the development of these stones, which are caused by an excess of lipid formation, and the latter, Beta Carotene Pro, an active form of vitamin A, has the capacity to provide an antioxidant effect that reduces the effects of free radicals.In addition to failing to effectively break up the

calcium oxalate crystal, this combo therapy prevents new calcium oxalate crystal deposition in the kidney. Finally, we came to the conclusion that beta-carotene and atorvastatin together have an antiurolithic impact on the body of the wistar rat model.

6.2 Future Research Direction

After Complete formation of study it is concluded that both drugs Atorvastatin and Beta Carotene in Combination form both are effective for urolithiasis but in future furrher studies on this combination form will provide a other activities of these two drugs regarding to urolithiasis and also benefical for other diseases in the body and In future this combination therapy will become the part of rationale use of drug for urolithiasis for their broad activities and actions of stone formation in the body .

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