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Biochemistry Letters

Effects of Zamzam water and mineral salts of treated Tap water on some biochemical parameters in male albino rats

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ABSTRACT

Background: Kidney stones are one of the most painful urologic disorders. Scientists have found evidence of kidney stones in a 7,000-year-old Egyptian mummy as they may contain various combinations of elements. Zamzam water showed highly significant readings in some inorganic elements, including Na, Ca, Mg, K, HCO_3, Cl, Fl, NO_3, and SO_4. The levels of these elements in Zamzam water may play a critical role in its effectives in the inhibition of calcium oxalate formation. ***Aim:*** This study was carried to investigate the effect of Zamzam water, and treated tab water with Ca, Mg, F, Cl and Na in adult male albino rats suffer from kidney stones induced by ethylene glycol. ***Materials & Methods:*** Kidney Calcium oxalate stones were induced in animals under study by oral administration of 0.75% of ethylene glycol dissolved in drinking water. Fifty rats were divided into equal 5 groups; each group was containing 10 rats: negative control, positive control (drinking tab water contains 0.75% ethylene glycol); negative Zamzam group (drinking Zamzam water only); Zamzam treated group (drinking Zamzam water contains 0.75% of ethylene glycol); and the last group tab water treated group (drinking treated water contain 0.75% ethylene glycol). After 4 weeks of treatment urine analysis and some biochemical analysis, were carried out in all studied groups. ***Results:*** we found that; significant differences in urine, Kidney functions and liver functions analysis in positive control group compared to negative control, while Zamzam group showed non-significance. Whereas Zamzam treated, showed a significant difference in all parameters compared to positive control group, more than tap water treated group. ***Conclusion:*** Zamzam water and treated tap water may play a critical role in its effectives in the inhibition of calcium oxalate formation.

INTRODUCTION

Zamzam water is located inside the Holy Mosque at about 20 meters east of the Ka'ba in Makkah Al-Mukarramah, Saudi Arabia. Zamzam water is different from other water in many ways, no bacteria can form at its source, it doesn't go mouldy nor does it change color, taste or smell. In Zamzam water well, there isn't any sign of biological growth [1]. This natural water has been found to be alkaline and rich in many minerals [2], which make it a potential agent. The Chemical analysis of Zamzam water contains some inorganic elements such as

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sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), bicarbonate (HCO_3), chloride (Cl), fluoride (Fl), nitrate (NO_{3-}), sulfate (SO_4), and other dissolved salts (TDS)[3]. Kidney stones are one of the most painful urologic disorders, have beset humans for centuries; as they may contain various combinations of chemicals. Kidney stones are formed when the urine is supersaturated with salt and minerals such as calcium oxalate, struvite (ammonium magnesium phosphate), uric acid and cysteine [4]. In most types of kidney stones, calcium oxalate crystals are found to be the main constituent. The prevalence of calcium oxalate crystals has been constantly increasing during past fifty years in industrialized as well as in developing countries. It is suspected that kidney stones have direct relationship to the composition of urine, which depends on the patients' habits [5]. About 75% of urinary stones are predominantly composed of calcium oxalate [6]. Urinary oxalate is considered a crucial risk factor for calcium oxalate stone formation. As the molar oxalate-to-calcium ratio is normally at 1:10, even slight changes in urinary oxalate concentration exert much larger effects on crystallization and stone formation than comparable changes in the calcium concentration[7].The problem of calculi formation is observed and reported in all parts of the urinary tract, the kidney, the ureter and the urinary bladder, which may considerably vary in size [8].There is no satisfactory drug available for use in clinical therapy then there is a need to explore more safe and cheap drugs from natural resources [9]. The aim of present study to use Zamzam water and treated tap water in the treatment of calcium oxalate stones induced by ethylene glycol.

MATERIALS AND METHODS

(I) Materials:

Zamzam water was obtained from (King Abdullah Bin AbdElaziz, Zamzam water for drinking), Saudi Arabia. Tap water was obtained from Nuclear Research Centre, Atomic Energy Authority. While, treated water was obtained by adding some mineral salts to tap water such as (Mg_2SO_4, Na_2CO_3, NaF, and $CaCl_2$) to have the same concentration in Zamzam water.

Chemicals: All chemicals used in these experiments were provided from Sigma Chemical Co. of high quality and purity.

(II) Animal Studies:

Animals' management: 50 adult male albino rats weighting (120-150 g) were provided from the National Research Centre, Dokki, Giza animals. The animals were maintained before experiment in controlled environment of temperature, humidity and light for 14 days in the animals' house of Nuclear Research Centre, Atomic Energy Authority under normal condition for adaption. They were fed on a commercial standard diet and tap water *ad libitum*.

Induction of Calcium oxalate kidney stones: Ethylene glycol was used to induce kidney stone formation by mixing (0.75% v\v) of ethylene glycol with different type of water of different groups for 28 days according to Velpandiani*et al.,* [10].

Experimental Design: Rats were divided according to their weight into equal 5 groups; each one includes 10 rats (each rat in separated metabolic cages). **Group 1 "G1":** drunk tap water only, served as the negative control; **Group 2 "G2":** drunk Zamzam water only; **Group 3 "G3":** drunk tab water contain (0.75 % v\v) ethylene glycol; **Group 4 "G4":** drunk Treated tap water contain some mineral salts mixed with (0.75% v\v) ethylene glycol; **Group 5 "G5":** drunk Zamzam water mixed with (0.75% v\v) ethylene glycol. The animals of all groups were maintained under the same conditions and were observed carefully every day for 28 days. At the end of the experiment, the 24 hours, collecting urine of the animals were collected for complete urine analysis,and blood samples were collected for biochemical assays (kidney and liver functions).

Complete Urine Analysis:After 28 days, urine 24 hours was collected from rats and studied for volume, pH value, and examined microscopically for crystals, according to **Free*etal.*,** [11]method.

Biochemical assays: At the end of experiment all rats were sacrificed and blood samples were collected for kidney function and liver function tests.

Kidney Function Tests: kidney function tests (urea and creatinine) were assayed according to methods of**Patton and Crouch** [12], **and Murray** [13]; respectively.

Liver Function Tests: liver functions (albumin, total proteins, Alanine aminotransferase "ALT", Aspartate aminotransferase "AST", and bilirubin) were assayed according to methods of **Doumas*et al.*, [14]Doumas *et al.*, [15], Schumann and Klauke[16], Karmen*et al.*, [17], and Dacie & Lewis** [18]; respectively.

Statistical Analysis: All results were analyzed by SPSS software (version 14). Data were expressed as mean ± SD. The student's t test was used for statistical analysis of differences between each two groups. Comparison of mean values of studied variables among different groups was done using ANOVA test. Pearson's correlation coefficient was used to quantify the relationship between the studied parameters. P<0.01 was considered to be significant [19].

RESULTS

Urine Analysis: Table (I) summarize the mean volume of collected urine, pH, and crystals. The mean urine volume was found to be 4.85 (ml) in negative control group, this volume was increase to be 7.97 (ml) in ethylene glycol group by 64%, (p<0.01), compared to negative control group. While, there was no significant difference between urine volumes in Zamzam water only "G2" group and negative control group. On Zamzam water and treated Tap Water treatments (G5, G4), urine volume were significantly decreased to be 5.5, and 5.4 (ml) by 32%, and 31%; respectively, (p<0.01), compared to ethylene glycol group (G3), *Fig. (1).* Urine pH was found as normal (acidic pH) in G1, there was no significant difference in Urine pH in other groups G2, G3, G4, and G5 compared to G1.

The microscopic examination of urine samples for the studied groups shows high density of calcium oxalate crystals in G3 compared to other groups. Ethylene glycol induced the formation of Ca-Oxalate crystals (+++) in urine sediment in G3, compared to G1. While, on Zamzam water and Treated Tap Water treatments (G5, G4), the formation of these crystals were reduced and their appearance in urine sediment were nil, *Fig. (2).*

Kidney function tests: Urea and Creatinine levels were found to be 31.60 ± 5.2 and 0.72 ± 0.105 (mg/dl) and 33.3±6.7 & 0.69±0.108; respectively in G1, and G2. A marked significant increase in urea and creatinine levels to 56.9±7.1 and 1.39±0.35 by 70.8% and 93.1%, (p<0.01) compared to negative control group. Meanwhile, Treated tap water "G4" showed a highly significant decrease in urea and creatinine levels to 38.44±9.2and 0.88±0.19, by -32.4%, and -36.7%, compared to Ethylene Glycol Group. Also, Zamzam water treatment Group "G5" showed a highly significant decrease in urea and creatinine levels to 36.13±8.0 and 0.75±0.06, by -36.5%, and -46.0%, compared to Ethylene Glycol Group, as shown in table (II), and Fig. (3).

Liver function tests: Our data reveled that, there were non-significant differences between mean levels of Total proteins, albumin, A/G Ratio and Bilirubin in G1, and G2. While, G3 showed a significant decrease in total protein, albumin from 5.7±0.36, 3.6±0.48 (g/dl) to 4.5±0.58, 3.3±0.63 by -21.1%, -8.33% and 3.1%, (p<0.01); but, slightly increased in A/G Ratio from 2.26±0.82 to 2.4±0.62 by 6.1%, and highly significant increase in Bilirubin to 1.0±0.07 (mg/dl) by 108.3% compared to negative control group. Meanwhile, Treated tap water "G4" and Zamzam water treatment Group "G5 showed a significant increase in total proteins and albumin levels to 5.01±0.59 & 3.5±0.16, and 4.97±0.30, 3.8±0.46 by 11.1%, & 6.06%, and by 10.44%, &15.15%; respectively compared to Ethylene Glycol Group "G3",

table (III), Fig. (3). Also, G4 and G5 showed a highly significant decrease in A/G ratio levels, and Bilirubin levels to 2.0±0.67 and 1.97±0.33, by -16.6%, and -17.9%, and to 0.45±0.05, and 0.43±0.09 by -55%, -57%; respectively compared to Ethylene Glycol Group, table (III), Fig. (4). ALT & AST activities showed a slightly significant increase by 13.4%, 6.1%; respectively in G3, compared to negative control group. While, Treated tap water "G4" and Zamzam water treatment "G5" groups showed a significant decrease in ALT, AST by -14.1%, -5.7%%, by -12.3%, -12.2%, respectively; compared to negative control group. As illustrated in table (III) and Fig. (4).

DISCUSSION:

Zamzam is natural water consumed by millions of Muslimsworldwide because oftheir religious beliefs.Water plays an important role in biological function [20] and Zamzam water is well known of its high conductivity [21]. In most types of kidney stones, calcium oxalate crystals are found to be the main constituent. The prevalence of calcium oxalate crystals has been constantly increasing during past fifty years.It is suspected that kidney stones have direct relationship to the composition of urine, which depends on the patients' habits [22].For the treatment and/or the prevention of calcium oxalate kidney stone formation, different approaches have been tested [23, 24]. Our present study aims to illustrate the effect of Zamzam water and some mineral salts in water on experimentally nephrotoxicity induced by ethylene glycol.

Our result revealed that, the urine volume/day was significantly increased in positive control by 64%, ($p<0.01$), compared to negative control group, as showed in table (I), Fig. (1).Also, ethylene glycol induced the formation of Ca-Oxalate crystals (+++) in the urine sediment in G3, compared to G1,. While, on Zamzam water and Treated Tap Water treatments (G5, G4), the formation of these crystals were reduced and their appearance in urine sediment were nil, table (I), Fig. (2). The administration of ethylene glycol (EG) caused a remarkable increase in urine output; it could be attributed to changes in threshold of tubular –reabsorption [25].

Sarmistha & Ramety [25], ***and Kumar et al.,*** [26] agreed with this study who postulated that the administration of ethylene glycol caused a remarkable increase in urinary output, and suggested that it was a result of impairment of kidney function. ***velpandian et al.,*** [10].disagreed with our study who postulated that the administration of ethylene glycol caused decrease in urinary output and suggested that it was a result of the obstruction of out flow of urine by stones in the urinary system. Acute overdose ingestions of EG can result in a renal failure that is linked with calcium oxalate monohydrate (COM) crystal accumulation in the kidney tissue [27].

The biochemical mechanisms for these processes are related to increases in the urinary concentration of calcium and oxalate. Stone formation in EG fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate [28]. Briefly stated the mode of action of EG is believed to be as follows: oxalic acid is one of the metabolites of EG, and can associate with calcium in renal tubule epithelium to form a precipitate. Renal tubule degeneration is believed to occur because the calcium oxalate monohydrate crystals can either cause biochemical damage to cells and/or physical obstructions to renal tubules with concomitant interstitial inflammation and stimulation of oxidative stress, which exacerbates the toxicity by impairing renal hemodynamics and urodynamic [29].

The injury is caused by calcium oxalate toxicity, could lead to additional cell injury [30]. When an injury has occurred to the glomerular or tubular cells in the kidneys, Transforming growth factor (TGF-β) secretion initially causes secondary infiltrating macrophages to invade the interstitial space.This tubule-interstitial inflammation is another key role for calcium oxalate during stone formation [31]. Zamzam water showed highly significant readings in

some inorganic elements, including Na, Ca, Mg, K, HCO_3, Cl, Fl, NO_3, and SO_4. The levels of these elements in Zamzam water may play a critical role in its effectives in the inhibition of calcium oxalate formation. Current evidence suggests that the consumption of diets low in calcium is associated with a higher overall risk for the development of kidney stones. This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointestinal tract [32, 33].The pH change could be attributed to the diuretic activity of Zamzam water. This diuretic effect may have an impact on stone precipitation and/or formation within the kidney through increasing the urine flow rates and consequently decreasing the stagnation of crystals within the renal tubules [5].

Magnesium could replace calcium ions affecting the dissolution of the later with a possible role played by pH. Magnesium decreases the urinary saturation of calcium oxalate by combining with urinary oxalate to form soluble magnesium oxalate so long as it is administered with meals this agreed with ***Parmer et al .,***who postulated that the magnesium inhibits formation of stones by binding to oxalate as forming soluble complex [34]. In addition, the presence of NaCl at a concentration as high as it seems in Zamzam water can affect the dissolution of such stone [5].G3 showed a highly significant increase in urea and creatinine levels by 70.8% and 93.1%, ($p<0.01$) compared to G1 Meanwhile, G4and G5showed a highly significant decrease in urea and creatinine levels by 32.4%, 36.7%, and 36.5%, and 46.0%, compared to G3, table (II), Fig. (3), and this agreed with ***Velpandlan et al.,***[10]***., Oliveira et al.,*** [27] ***and Parmer et al.,***[34] who postulated that the administration of ethylene glycol caused a remarkable increase in urea and creatinine levels as result of oxidative stress of ethylene glycol in the kidney. Kidney is vital in maintenance of homeostasis through the excretion of catabolites like urea, creatinine, uric acid and elevated concentration of these indicates compromised renal function.

In response to EG treatment, urea, creatinine were increased, suggesting an impairment of kidney functions. These effects could also be attributed to the changes in the threshold of tubular re-absorption, renal blood flow and glomerular filtration rate [35]. EG is known to decrease the glomerular filtration rate due to the obstruction to the flow of urine by stones in urinary system. Due to this, the waste product, particularly nitrogenous substances such as urea, creatinine gets accumulated in blood. The increased serum level of creatinine can be attributed to the damaged nephron structural integrity. Pathologic studies have shown that the renal failure from EG is associated with proximal tubule cell necrosis leading to production of several metabolites and accumulation of large calcium oxalate monohydrate crystals in tubular lumen [36].

G3 showed a significant decrease in total protein, albumin from by 21.1%, 8.33% and 3.1%, ($p<0.01$). Meanwhile, G4 and G5 showed a significant increase in Total proteins, albumin levels by 11.1%, & 6.-6%, and by 10.44%, &15.15%; respectively compared to G3, table (III), Fig. (4).ALT & AST activities showed a slightly significant increase by 13.4%, 6.1%; respectively in G3, compared to negative control group. While, G4 and G5 groups showed a significant decrease in ALT, AST by -14.1%, -5.7%%, by -12.3%, -12.2%, respectively; compared to negative control group.and this agreed with ***Al-Attar*** [23], who postulated that the administration of ethylene glycol caused a remarkable effect on liver function tests as result of strong indication of hepatic impairment and as result of hepatotoxicity of ethylene glycol [23].

Liver is the target organ for the metabolism of all drugs and toxic chemicals. Any drug administered will exert its action at the hepatic level. Hence an evaluation of the hepatic function has been carried out in this study as well by estimating the alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Total bilirubin as they are released into blood following hepatocellular damage [37, 38, 39].

Conclusion:

In conclusion drinking Zamzam water and treated tap water with some mineral salts decrease the formation of kidney stones as it riches in many minerals which make it a potential agent.

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Table (I): effect of Zamzam and treated tap water on Urine Volume in all studied groups:

Variables	Negative Control Group "G1"		Zamzam Water group "G2"		Ethylene Glycol Group "G3"		Treated tap water +Ethylene Group "G4"		Zamzam water +Ethylene Group "G5"	
	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change	Mean ± SD.	% Change
Urine Volume (ml/day)	4.85±0.35	-----	4.95±0.27	2.0%	7.97±0.47	64.3%	5.46±0.02	-31.49%	5.38±0.21	-32.49%
pH	Acidic		Acidic		Acidic		Acidic		Acidic	
Ca-Oxalates	Amp.Urates (Few)		Amp.Urates (Few)		Ca-Oxalate (+++)		Ca-Oxalate+ Urates (Few)		Ca-Oxalate (Nil)	

Table (II): Effect of Zamzam and treated tap water on kidney function tests in all studied groups:

Group		Urea (mg/dl)	Creatinine (mg/dl)	P
Negative control "G1"group	Mean ±SD	31.60 ± 5.2	0.72 ± 0.105	----
	%change	-----	-------	
Zamzam Water group "G2"	Mean ±SD	33.3±6.7	0.69±0.108	p>0.01
	%change	5.3%	-4.1%	
Ethylene Glycol Group "G3"	Mean ±SD	56.9±7.1	1.39±0.35	p<0.01
	%change	70.8%	93.1%	
Treated tap water +Ethylene Group "G4"	Mean ±SD	38.44±9.2	0.88±0.19	p<0.01
	%change	-32.4%	-36.7%	
Zamzam water +Ethylene Group "G5"	Mean ±SD	36.13±8.0	0.75±0.06	p<0.01
	%change	-36.5%	-46.0%	

Table (III): Effect of Zamzam and treated tap water on Liver function tests in all studied groups:

Group		ALT(U/l)	AST (U/l)	T.P. "g/dl"	Albumin "g/dl"	A/G ratio	T. Bili. " mg/dl"
Negative control "G1"group	Mean ±SD	80.6±5.4	93.7±7.7	5.7±0.36	3.6±0.48	2.26±0.82	0.48±0.12
	%change	-----	------	-----	-----	-----	-----
Zamzam Water group "G2"	Mean ±SD	80.5±3.02	89.6±10.7	5.4±0.74	3.4±0.28	2.7±0.93	0.49±0.11
	%change	-.12%	-4.3%	-5.2%	-5.5%	22.7%	2.0%
Ethylene Glycol Group "G3"	Mean ±SD	91.4±3.2	99.5±6.5	4.5±0.58	3.3±0.63	2.4±0.62	1.0±0.07
	%change	13.4%	6.1%	-21.1%	-8.33%	6.1%	108.3%
Treated tap water +Ethylene Group "G4"	Mean ±SD	78.5±5.2	93.8±2.6	5.01±0.59	3.5±0.16	2.0±0.67	0.45±0.05
	%change	-14.1%	-5.7%%	11.1%	6.06%	-16.6%	-55%
Zamzam water +Ethylene Group "G5"	Mean ±SD	80.2±5.8	87.4±8.8	4.97±0.30	3.8±0.46	1.97±0.33	0.43±0.09
	%change	-12.3%	-12.2%	10.44%	15.15%	-17.9%	-57%

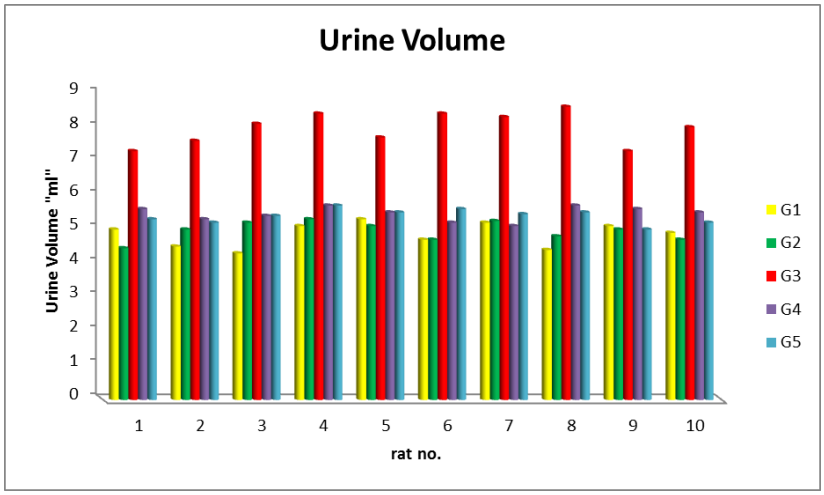

Fig. (1): Urine Volume in all studied groups (ml).

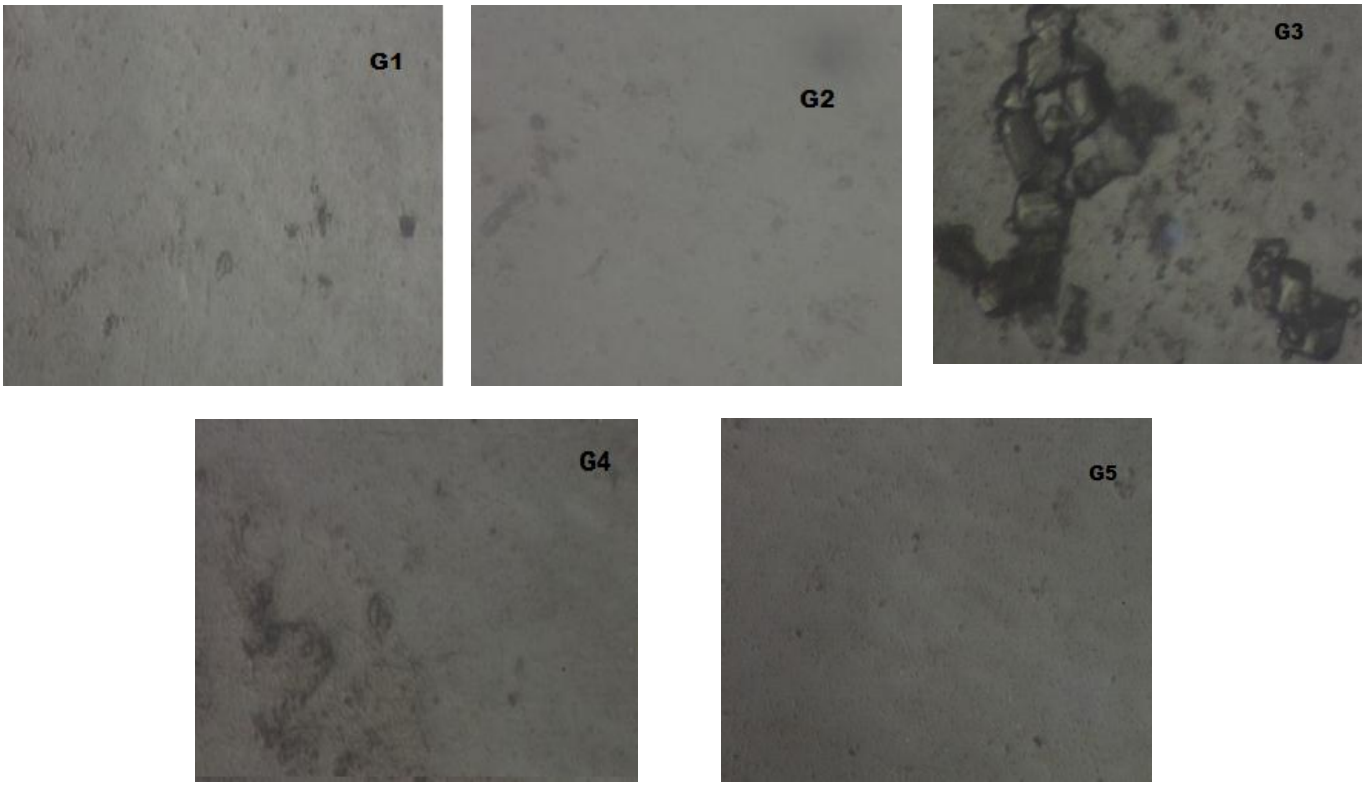

Fig. (2): Urine Crystals in all studied groups. ***G1, G2:*** microscopic examination showed appearance of Amp.Urates (Few). ***G3:*** Ethylene glycol group, appearance of Ca-Oxalate crystals (+++) in urine sediment. ***G4, G5:*** reduction of Ca-oxalate crystals and their appearance in urine sediment were nil.

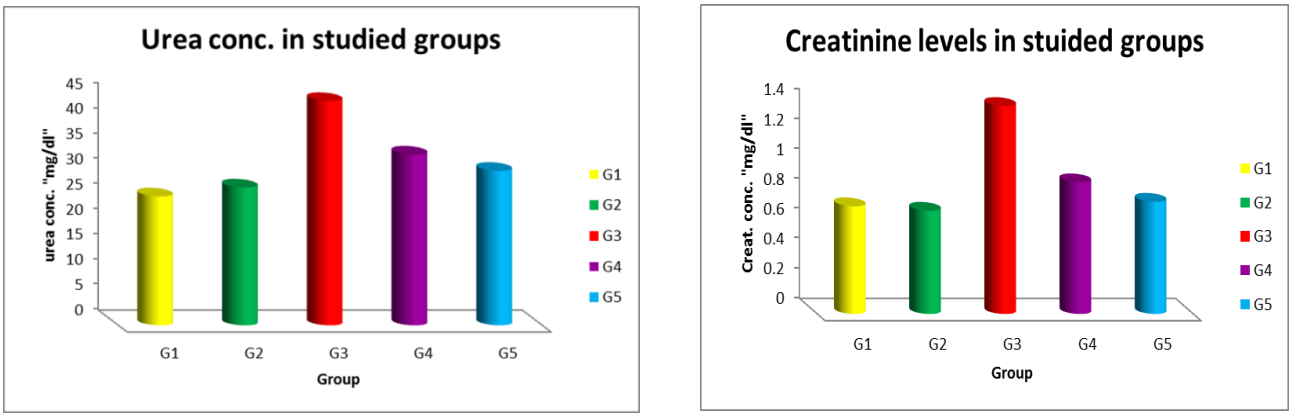

Fig. (3): Kidney function tests in all studied groups.

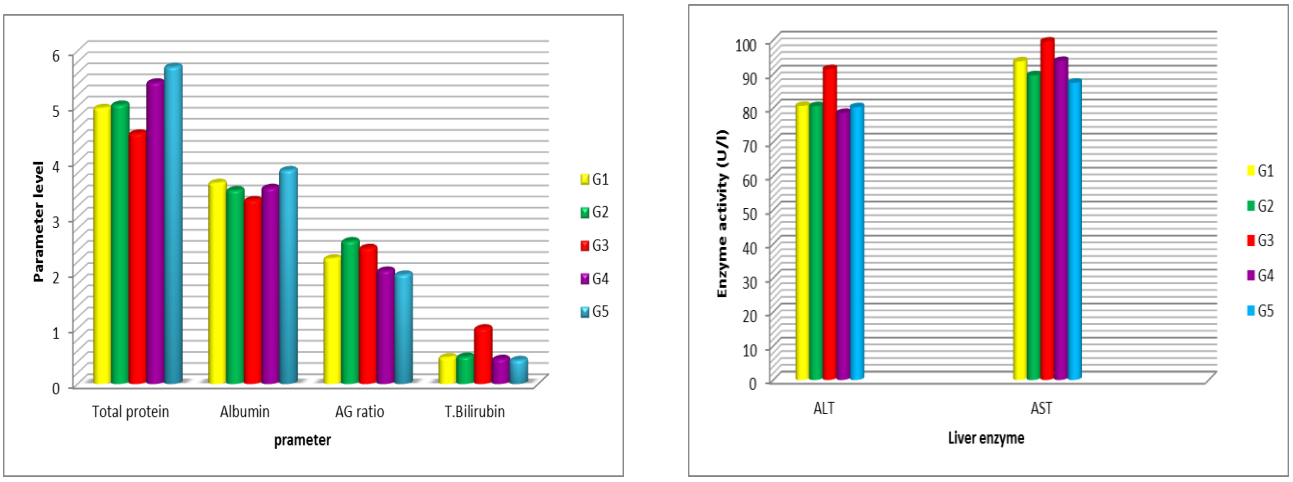

Fig. (4): Liver Function tests in all studied groups.