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CLINICAL EVALUATION OF THE EFFICACY OF PYRIDOXAL-5'-PHOSPHATE AND MAGNESIUM IN PREVENTION OF GLYCINE INDUCED UROLITHIASIS IN MALE RATS

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Abstract- The objective of the present study was to assess the efficacy of high level of pyridoxal-5'-phosphate (PLP) and magnesium in the form of magnesium oxide (MgO) at low and high levels in the prevention of urolithiasis induced by high dose of glycine in male albino rats. Seven groups each of ten adult male Albino rats (Sprague-Dawely) strain, mean weight varied between 97.8 to 100.2 g were fed on either basal control diet or experimental diets containing high level of glycine (3%) to induce urolithiasis and supplemented with PLP at high dose (0.7%) alone or incorporated with MgO at two levels (0.25% and 0.5%) throughout two successive experimental periods (15 and 30 days). 24h urine samples and serum were analyzed for biochemical indicators of kidney function. The results showed that the supplementation of both PLP and MgO to the urolithic rats decreased the urinary excretion of oxalate, calcium, phosphates, uric acid, urea, and total proteins and increased the excretion of magnesium and citrates. Serum levels of creatinine, albumin, total cholesterol, potassium, sodium, PTH, phosphates, and ALP activity were decreased and the activities of LDH and G6PD were increased in treated rats by PLP and MgO at high doses throughout two successive experimental periods. The present study concluded that, urolithiasis and renal calcium oxalate deposition induced by high dose of glycine (3%) in male albino rats could be completely prevented by diets high in magnesium (0.5%) and partially prevented by very high dietary levels of PLP (0.7%). The combined supplementation of PLP and Mg to experimental rats improved urinary functions and increased the excretion of urinary citrate and magnesium more than either supplement alone.

Keywords- Urolithiasis, Glycine, Magnesium Oxide, Pyridoxal-5'-Phosphate

Abbreviations- CaOx, calcium oxalate; GAD, glycolic acid dehydrogenase; GAO, glycolic acid oxidase; MgO, magnesium oxide; PLP, pyridoxal-5'-phosphate

Introduction

Urolithiasis is the condition where urinary calculi are formed or located anywhere in the urinary system [1]. Urinary calculi are the third prevalent disorder in the urinary system [2]. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate [3]. Urinary calculi may cause obstruction, infection and hemorrhage in the urinary tract system. Oxalic acid, an end product of metabolism when produced in large amounts in the body is known to produce calcium oxalate urinary stones, both in animals and humans [4]. Urinary stones result from biochemical changes which disturb the physicochemical equilibrium in normal urine by increasing the urinary concentration of lithogenic substances like calcium, oxalate and phosphate or lowering the inhibitors of crystallization like citrate and magnesium [5].

It has been demonstrated that feeding a high level of glycine increase urinary oxalate both in normal subjects and in patients with primary hyperoxaluria [6]. Glycine is abundantly present in nearly all animal foods and constitutes 25% of the amino acid content of collagen. If glycine is converted to oxalate, an increased intake of high glycine foods may increase the risk of kidney stone disease because a slight increase in urinary oxalate is a very sensitive index of calcium stone formation [7].

Because of the successful treatment of some individuals with primary hyperoxaluria type 1 (PH1) with pyridoxine to reduce their urinary oxalate excretion, several studies have examined whether pyridoxine would be beneficial in the treatment of idiopathic calcium oxalate stone disease by decreasing urinary oxalate excretion [8].

It has been reported that magnesium consumption did not influence stone formation in their male and female cohorts [5]. Some investigators have reported decreased urinary magnesium excretion in stone formers [9]. In vitro studies have demonstrated that magnesium inhibits calcium oxalate and calcium phosphate crystal nucleation, growth, and aggregation. Magnesium forms a soluble complex with oxalate in urine and binds oxalate

in the gut [10]. Magnesium reduces urinary calcium oxalate (CaOx) saturation by forming magnesium oxalate, which is more soluble than CaOx in the urinary tract [11].

Materials and Methods

Materials

Glycine (NH2CH2COOH), Pyridoxal-5'-Phosphate (PLP), and magnesium oxide (MgO) were brought from EL-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt.

Experimental animals

Seven groups each of ten adult male Albino rats (Sprague-Dawely) strain, mean weight varied between 97.8 to 100.2 g were used. They were obtained from Helwan breeding farm, Cairo, Egypt. The animals were divided into seven homogenous groups and housed individually in metabolic cages with wire mesh bottoms and maintained in temperature 25□C □ 5□C, humidity 50% □ 10% and light dark cycle held constant 12/12 h. During the conditioning periods (15 and 30 days) and throughout the experiment, food and water were provided ad libitum.

Experimental diets

Seven balanced diets were used in the present study illustrated in table (1).

Collection and Analysis of Urine

All animals were kept in individual metabolic cages and 24h urine samples were collected on 15 and 30 days of experiment. The preparation of samples was carried out immediately after urine collection and analyzed for urinary parameters. Urine samples were acidified with 10% hydrochloric acid to block the growth of bacteria and molds and stored below 4oC for subsequent analysis. Urinary pH was measured using a pH meter (Beckman coulter). Urine was also analyzed for chloride, magnesium, calcium, oxalate, citrate, phosphate, uric acid, urea, and total proteins.

Serum Analysis

At the end of the experiment (15 and 30 days), rats were fasted for 12 hrs, then the animals were scarified under ether anesthesia and blood samples were taken from hepatic portal vein in centrifuge tubes. The blood sample was left for 15 minutes at room temperature then centrifuged at 10000 rpm for 20 minutes to provide the serum for biochemical analysis. Serum was analyzed for lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) activities and also for calcium, phosphate, parathyroid hormone (PTH), creatinine, albumin, total cholesterol, potassium, and sodium.

Liver Analysis

Liver was separated, rinsed and washed by saline solution (NaCl 0.9%), then blotted on filter paper. A known amount of liver was weighed and homogenized in 0.01 tris-HCl buffer, pH 7.4 to get a homogenate. Glucose-6-phosphate dehydrogenase (G6PD) activity was determined by enzymatic kinetic method kit developed by Sigma diagnostic, USA.

Statistical Analysis

The data were statistically analyzed by SPSS version 10.0 statistical packages. Data were presented as a mean ± SD; statistical differences between groups were performed using t-test. Differences considered significant when $p<0.01$.

Results

Effects on urinary pH, magnesium calcium levels and Ca/Mg ratio

Urinary pH value was decreased significantly ($p<0.01$) in renal stone induced rats (G2) throughout the two experimental periods (15 and 30 days) when compared to the control group. An observed increase in the urinary pH was recorded in all the tested rats which fed on diets supplemented with PLP alone or in combination with magnesium when compared with G2. The results of the present study showed that the urinary calcium excretion was increased significantly ($p<0.01$) in glycine induced group (G2) and gradually decreased by magnesium oxide and PLP supplementation. However, the urinary Mg+2 concentrations were decreased in renal stone induced group (G2) when compared to control group. Supplementations with PLP and Mg in combination significantly prevented these changes and restore the values to near normal value after 15 and 30 days of the experiment. The Ca/Mg ratio was increased significantly ($p<0.01$) during both periods of the study, but more so during the latter period in renal stone induced group (Table 2).

Effects on the urinary excretion of oxalate, citrate, phosphate and chloride

Glycine feeding resulted in a significant increase in urinary oxalate and phosphate excretion as compared to control (G1). Supplementation of PLP alone or with magnesium at two levels (0.25% and 0.5%) significantly decreased oxalate and phosphate excretion. Feeding of diets containing glycine only to induce urolithiasis for a period of 30 days produced almost 2.9 fold decrease in urinary citrate excretion as compared to the basal value. Whereas, simultaneous supplementation of Pyridoxal-5'-Phosphate and magnesium oxide at a high dose 0.5% (G7) raised urinary citrate by about 3.1 fold as compared to renal stone induced rats throughout the second experimental period (30days). There are no significant differences in the value of urinary chloride among all the experimental groups throughout the two periods (Table 3).

Effects on the urinary uric acid, urea, and total proteins levels

Renal stone induction caused significant increase in urinary uric acid, urea, and total proteins excretion (G2) which was dose-dependent prevented in rats treated with magnesium and PLP. Pyridoxal-5'-Phosphate supplementation at a high dose (0.7%) had significant ($p<0.01$) improving effect on these urinary parameters levels especially when combined with magnesium at a high dose (0.5%) throughout the two experimental periods (table 4).

Effects on serum calcium, phosphate, and PTH values

The concentration of calcium and phosphate were significantly ($p<0.01$) increased in renal stone induced group (G2) at 15 and 30 days when compared to the control group. Addition of PLP and magnesium at a level 0.5% improved the levels of these parameters when compared with G2. Feeding diets supplemented with magnesium at high dose (0.5%) were modulate the serum calcium values in rats groups (G5 and G7) than that rats groups (G4 and G6) fed diets containing low dose of magnesium (0.25%). Serum concentration of PTH was elevated in the lithogenic rats when compared to control group throughout the first 15 days and second periods of experiment (30 days). The addition of PLP in combination with magnesium (G6 and G7) improved significantly the serum levels of phosphate and PTH when compared to groups supplemented with magnesium alone (Table 5).

Effects on serum levels of creatinine, albumin, total cholesterol, sodium, and potassium

Serum concentration of total cholesterol and albumin were elevated significantly ($p<0.01$) in the lithogenic rats when compared to control group throughout the first (15 days) and second periods of experiment (30 days). Serum creatinine, sodium, and potassium concentrations were significantly higher in G2 and gradually decreased in other groups by adding PLP and magnesium at two levels. Supplementation with PLP and magnesium significantly modulated these changes and restored it to near control value. Rats fed on high dose of magnesium (0.5%) alone (G5) or in combination with PLP (G7) have highly significant improvement ($p<0.01$) than that rats groups (G4 and G6) fed diets containing low dose of magnesium (0.25%) (Table 6).

Effects on ALP, LDH, and G6PD activities

Addition of glycine at a high dose (3%) reduced the activity of G6PD and LDH enzymes. While the activity of ALP tended to be increased in lithogenic rats group (G2) throughout the experimental periods (15 and 30 days). The enzyme activities were significantly improved in rats groups fed diets supplemented with PLP and Mg at two levels in combination when compared to renal stone induced group (Table 7).

Discussion

Urolithiasis is a common disease with an increasing incidence and prevalence worldwide. Lifestyle and dietary choices implicated in the complex of the metabolic syndrome are important factors contributing to such developments [1]. The salts and acids that normally crystallize in kidney stones do so because of their relative insolubility in urine. The most insoluble is calcium oxalate (CaOx); once a CaOx stone is trapped in the urinary tract, it is almost impossible for it to re-dissolve. Its solubility is independent of urinary pH, unlike the solubilities of other common stone constituents such as uric acid (soluble in alkali) or calcium phosphate (CaP) and magnesium ammonium phosphate (soluble in acid). Uric acid crystallizes out at pH<5.4, but it will re-dissolve slowly at values higher than 6.2. The results of the present study showed that, the urinary pH was decreased and reached to 5.9 and 5.7 in renal stone induced rats throughout the two experimental periods 15 and 30 days respectively. An observed increase in the urinary pH was recorded in all the tested rats which fed on diets supplemented with pyridoxal-5'-phosphate alone or in combination with magnesium.

The urinary calcium excretion was increased significantly by glycine induction and gradually decreased by magnesium and PLP supplementation. A possible mechanism for an effect of MgO and PLP supplementation in protecting against calcium oxalate urolithiasis might involve an effect on the solvent characteristics of urine. Tseng et al reported that a variety of metabolites found in urine are effective in increasing the solubility of calcium phosphate and calcium oxalate in urine. Citrate and magnesium are particularly effective as solubilizers [12].

Hypocitraturia is considered a correctable cause of calcium urolithiasis and occurs in 19% to 63% of patients with urolithiasis [13]. Hypomagnesuria does not usually present alone, and most patients with hypomagnesuria also have hypocitraturia. The causes of hypomagnesuria are inflammatory bowel disease associated with malabsorption [14]. Our results are in agreement with the findings of Zerwekh et al who reported that, MgO improved urinary parameters among hypocitraturic patients [15].

A high urinary Ca/Mg ratio in association with hyperoxaluria has been proposed to be causally related to the formation of kidney stones. Magnesium supplementation was associated with a potentially beneficial alteration in this regard. The results of the present study showed that there was an MgO-induced decrease in the urinary Ca/Mg ratio for the 24h urine throughout two periods compared with the ratios observed for the control treatment. Pyridoxal-5'-phosphate was nearly as effective as MgO in terms of reducing urinary oxalate excretion and was associated with a reduction in the urinary Ca/Mg ratio. The primary mechanism by which 0.5% of magnesium consumed with 0.7% PLP can decrease urinary oxalate is by decreasing absorption. The present study was unique in that it involved a direct comparison of the relative abilities of pyridoxal-5'-phosphate and magnesium to reduce oxalate absorption and urinary excretion.

Numerous studies have contributed to the elucidation of the metabolic pathways for the conversion of glycine to oxalic acid in mammals. The principal known immediate precursor of oxalic acid is glyoxylic acid [16]. Administration of glycine at a dose of 3% to adult male rats for a period of 15 and 30 days resulted in marked hyperoxaluria due to conversion of glycolate to oxalate in the body. Urinary supersaturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in

glycine fed is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate [17].

Supplementation of PLP to glycine treated rats resulted in a significant decrease in urinary oxalate levels. The decreased synthesis of oxalate from glycolate in pyridoxine fed rats can be explained on the basis of decreased activities of both glycolic acid oxidase (GAO) and glycolic acid dehydrogenase (GAD) enzymes in these animals [18]. Glycolate is known to freely enter into peroxisomes where it is metabolized by GAO to oxalate with glyoxylate as an intermediate. The mechanism of pyridoxine inhibition of GAO has been proposed to be mediated via the androgens. Pyridoxal phosphate regulates the translocation of steroid hormones in liver, thus preventing its nuclear translocation and binding [19]. Decreased translocation of steroid-receptor complex to the nuclei in pyridoxine excess could be the regulating factor of GAO activity. Glycolic acid dehydrogenase, an important enzyme of oxalate biosynthesis in conditions of glycolate excess is located in hepatic cytosol and is known to be dependent on the pyridoxine status of the rats. Supplementation of PLP to glycine fed rats besides lowering urinary oxalate also produced a significant increase in the excretion of citrate.

The urinary citrate is known to increase the solubility of calcium oxalate in urine and also inhibit calcium oxalate crystal growth, thereby retarding calcium oxalate stone formation [20]. Thus pyridoxal-5'-phosphate prevents the glycolate induced hyperoxaluria by regulating the endogenous synthesis of oxalate through inhibition of oxalate synthesizing enzymes of the liver. Most calcium stones are composed predominantly of calcium oxalate (CaOx) with small amounts of calcium phosphate (CaP). Stones that contain more than 50% CaP are uncommon and form when urinary CaP supersaturation is persistently elevated. The major determinants of CaP supersaturation alkaline urine combined with hypercalciuria. This condition is seen in patients who have distal renal tubular acidosis, whether genetic or acquired. CaP stones are associated with a more destructive renal pathology [21].

An increase in urinary phosphate excretion was observed in glycine induced urolithic rats. Increased excretion of phosphate has been reported in stone formers [22]. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition [17]. In addition, the increased levels of alkaline phosphates in urolithic rats might be due to increased basal metabolic rate [22]. The results of the present study showed that, the supplementation of PLP and Mg in combination lowered the excretion of phosphates and reduces the risk of stone formation.

Urolithiasis is a common urologic disease that affects approximately 10% of the population worldwide [23]. Uric acid is a frequent component of urinary stones and affects calcium stone formation. The prevalence of uric acid stones is estimated to be 5–10% of all urinary stone diseases [24]. The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric rats. Uric acid interferes with calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminoglycans. The predominance of uric acid crystals in calcium oxalate stones suggests its primary role in stone formation [25].

The results of the present study showed increased protein excretion in glycine induced urolithic rats. Proteinuria reflects proximal tubular dysfunction. Supersaturation of urinary colloids results in precipitation as crystal initiation particle which when trapped acts as a source leading to subsequent crystal growth. Supplementation of PLP and Mg had powerful effects on minimizing the excretion of protein and thus might have prevented the stone formation.

It was suggested that the development of nephrocalcinosis related to high glycine diets might be related to elevated PTH concentration. In addition, it has been also demonstrated that PTH increased the renal tubular reabsorption of calcium and decreased that of phosphorus [26]. If the concentration of calcium in the renal tubules and cells are increased by this mechanism, the presence of excessive amount of glycine will results in the precipitation of calcium phosphate in the kidney. Nephrocalcinosis is a disorder involving an intracellular and intratubular deposition of calcium phosphate [27]. Severe renal calcification with cellular degeneration caused that response for PTH were declined. The increase of serum PTH concentration might also cause an increase in the intratubular phosphate concentration at the corticomedullary junction, which in turn might trigger intracellular and/or intratubular calcification. Increasing urinary excretion of phosphate was directly caused by glycine supplementation, which also resulted in increasing of urinary excretion of calcium [26].

The results of the present study showed that magnesium supplementation in the diet had a protective effect against renal calcification. Robert reported, the serum PTH was low or non-detectable in spite of hypocalcaemia in a patient with chronic magnesium deficiency, and the administration of magnesium in the diet led to parallel increases with serum PTH [28].

In calculi-induced rats, marked renal damage was seen as indicated by the elevated serum levels of creatinine and albumin which are markers of glomerular and tubular damage. Supplementation of PLP and Mg at high doses showed to prevent the elevation of serum levels of these markers and inhibits the urolithiasis process. Therefore, may prevent calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria, which in turn can prevent calcium oxalate crystal attachment and subsequent development of kidney stones [29].

Increased serum concentration of cholesterol is the major lipid abnormality in the nephrotic syndrome in man. Serum total cholesterol was found to be raised in the stone forming rats. Similar increase in serum cholesterol has been observed in pyridoxine deficient rats and renal stone patients. In our study PLP alone or in combination with Mg were reduced serum total cholesterol

concentration significantly. Van Camp et al reported, a high phosphorus diet and low magnesium diet induced an increase in urinary albumin excretion [30]. In the present study, urinary albumin excretion was decreased by increased magnesium intake (0.5%). Increases in urinary albumin excretion may be due to the obstruction of proximal tubular albumin reabsorption, since albumin reabsorption occurs mainly in the proximal tubule. The results suggest that increasing the magnesium intake prevented the depression of proximal tubular function due to 3% glycine diet. Then urinary albumin excretion may be decreased by increased magnesium intake, leading to albumin reabsorption in the proximal tubule. The results in the present study showed that the feeding of glycine to control rats significantly reduced the levels of LDH and G6PD. While the activity of ALP tended to be increased in lithogenic rats. In vitro studies, oxalic acid inhibited LDH and G6PD to the same extent. The low activities of LDH and G6PD observed in urolithic rats may be due to inhibition by the oxalate or glycolate of their livers.

In conclusion, urolithiasis and renal calcium oxalate deposition induced by high dose of glycine could be completely prevented by diets high in magnesium (0.5%) and partially prevented by high dietary dose of PLP (0.7%). The combined supplementation of PLP and Mg to experimental rats improved urinary functions and increased the excretion of urinary citrate and magnesium more than either supplement alone.

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Table 1: The composition of each experimental diet (g/100 g diet)

Component	G1	G2	G3	G4	G5	G6	G7
Corn Starch	65	62	61.3	61.75	61.5	61.05	60.8
Casein	15	15	15	15	15	15	15
Corn oil	10	10	10	10	10	10	10
Non-nutritive cellulose	5	5	5	5	5	5	5
Vitamin Mix.	1	1	1	1	1	1	1
Salt mix.	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Choline chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
DL-Methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Glycine	-	3	3	3	3	3	3
Pyridoxal-5'-Phosphate	-	-	0.7	-	-	0.7	0.7
Magnesium Oxide	-	-	-	0.25	0.5	0.25	0.5

Table 2: Effect of different experimental diets on urinary pH, calcium, magnesium levels and Ca/Mg ratio

Groups	Days	pH	Calcium (Ca) (mg/24h)	Magnesium (Mg) (mg/24h)	Ca/Mg ratio
G1	15	6.18 ± 0.2^{ac}	0.283 ± 0.01^{a}	0.661 ± 0.08^{a}	0.428 ± 0.03^{a}
	30	$6.17\pm0.3^{1,3}$	0.292 ± 0.02^{1}	0.660 ± 0.07^{1}	0.442 ± 0.04^{1}
G2	15	5.96 ± 0.1^{b}	0.597 ± 0.03^{b}	0.230 ± 0.03^{b}	2.595 ± 0.14^{b}
	30	5.79 ± 0.2^{2}	0.925 ± 0.08^{2}	0.121 ± 0.05^{2}	7.64 ± 0.45^{2}
G3	15	6.31 ± 0.2^{a}	0.396 ± 0.02^{cde}	0.539 ± 0.07^{c}	0.734 ± 0.06^{c}
	30	6.29 ± 0.3^{1}	0.452 ± 0.03^{3}	0.501 ± 0.06^{3}	0.902 ± 0.08^{3}
G4	15	5.99 ± 0.3^{bc}	0.604 ± 0.05^{bc}	0.873 ± 0.08^{d}	0.691 ± 0.05^{c}
	30	5.98 ± 0.5^{2}	0.731 ± 0.06^{4}	0.691 ± 0.05^{4}	1.057 ± 0.11^{4}
G5	15	6.12 ± 0.1^{ab}	0.313 ± 0.03^{a}	0.925 ± 0.09^{e}	0.338 ± 0.02^{d}
	30	$6.10\pm0.2^{2,3}$	$0.272\pm0.02^{5,6}$	1.839 ± 0.03^{5}	0.147 ± 0.01^{5}
G6	15	6.28 ± 0.1^{a}	0.429 ± 0.03^{d}	0.877 ± 0.12^{d}	0.489 ± 0.05^{a}
	30	6.30 ± 0.1^{1}	$0.318\pm0.02^{1,5}$	0.929 ± 0.14^{6}	0.342 ± 0.02^{6}
G7	15	6.22 ± 0.1^{a}	0.305 ± 0.02^{ae}	1.889 ± 0.09^{f}	0.161 ± 0.01^{e}
	30	$6.20\pm0.1^{1,3}$	$0.290\pm0.01^{1,6}$	1.901 ± 0.13^{7}	0.152 ± 0.02^{5}

Table 3: Effect of different experimental diets on urinary oxalates, citrates, phosphates and chlorides

Groups	Days	Oxalates (mg/24h)	Citrates (mg/24h)	Phosphates (mg/24h)	Chlorides N.S (mmol/24h)
G1	15	0.22±0.02[a]	2.99±0.32[a]	6.78±0.30[ac]	149.0±3.1
	30	0.25±0.02[1]	2.87±0.23[1]	6.75±0.21[1,3]	149.2±4.2
G2	15	0.90±0.11[b]	1.21±0.12[b]	7.73±0.11[b]	150.1±6.2
	30	1.39±0.32[2]	0.99±0.06[2]	8.55±0.42[2]	151.3±5.3
G3	15	0.28±0.01[c]	2.11±0.20[c]	7.01±0.39[ad]	150.0±4.3
	30	0.35±0.03[3]	1.89±0.12[3]	7.22±0.23[1,4]	149.3±4.2
G4	15	0.72±0.06[d]	1.49±0.09[b]	7.11±0.22[cdef]	150.2±3.4
	30	0.80±0.07[4]	1.02±0.08[2]	7.52±0.40[3,4,5,6]	151.7±2.5
G5	15	0.26±0.02[e]	2.64±0.20[d]	6.92±0.21[a]	149.1±3.3
	30	0.29±0.01[5]	2.13±0.2[3,4]	7.01±0.11[1,5]	150.2±5.2
G6	15	0.32±0.02[f]	2.23±0.20[c]	6.99±0.27[ae]	149.9±5.1
	30	0.28±0.01[5]	2.20±0.20[4]	7.21±0.25[1,6]	148.7±4.2
G7	15	0.18±0.01[g]	2.92±0.21[a]	6.82±0.26[af]	148.2±3.9
	30	0.20±0.01[6]	3.11±0.30[1]	6.55±0.19[1]	149.3±4.6

- N.S. =Non Significance

Table: 4: Effect of different experimental diets on urinary uric acid, urea, and total proteins levels

Groups	Days	Uric Acid (mg/24h)	Urea (mg/24h)	Total Proteins (mg/24h)
G1	15	1.55±0.10[ab]	695.8±9.12[a]	6.29±0.73[a]
	30	1.52±0.11[1]	693.9±9.3[1]	6.22±0.81[1]
G2	15	2.12±0.12[c]	815.3±12.2[b]	9.22±0.92[b]
	30	2.52±0.32[2]	878.2±13.7[2]	10.53±0.82[2]
G3	15	1.73±0.12[de]	762.7±12.5[c]	7.12±0.62[c,d]
	30	2.11±0.23[3]	822.1±15.2[3]	8.21±0.92[3]
G4	15	2.08±0.29[c]	799.5±11.7[b]	8.20±0.53[e]
	30	2.12±0.27[3]	850.1±17.3[4]	9.37±0.61[4]
G5	15	1.62±0.20[ad]	701.4±15.1[a]	7.73±0.60[ce]
	30	1.76±0.21[4]	705.3±14.1[1]	7.22±0.53[3]
G6	15	1.69±0.09[ae]	750.9±19.1[c]	7.12±0.71c
	30	1.72±0.08[4]	742.7±18.2[5]	7.18±0.53[3]
G7	15	1.47±0.19[b]	682.8±8.2[a]	6.79±0.39[ad]
	30	1.50±0.15[1]	690.2±7.1[1]	6.99±0.65[1,3]

Table 5: Effect of different experimental diets on serum calcium, phosphates and PTH levels

Groups	Days	Calcium (Ca^{+2}) (mmol/L)	Phosphates (mmol/L)	PTH (Pg/ml)
G1	15	3.72±0.12[a]	1.67±0.09[a]	0.83±0.006[a]
	30	3.77±0.19[1]	1.62±0.11[1]	0.82±0.005[1]
G2	15	4.31±0.29[b]	3.21±0.13[b]	1.92±0.03[b]
	30	5.22±0.21[2]	3.62±0.23[2]	2.17±0.05[2]
G3	15	3.90±0.12[c]	1.85±0.08[cd]	1.89±0.07[b]
	30	4.21±0.12[3]	1.92±0.08[3,4]	0.92±0.009[3]
G4	15	4.11±0.13d	2.28±0.03[e]	1.91±0.04[b]
	30	4.90±0.15[4]	2.01±0.02[3]	1.70±0.002[2]
G5	15	3.66±0.31[e]	1.74±0.01[a]	1.35±0.01[c]
	30	3.62±0.22[5]	1.52±0.09[1]	0.96±0.009[4]
G6	15	3.87±0.32[c]	1.82±0.01[ac]	0.99±0.008[d]
	30	3.71±0.21[1,5]	1.70±0.03[1,4]	0.81±0.007[1]
G7	15	3.55±0.31[e]	1.73±0.02[ad]	0.91±0.009[e]
	30	3.42±0.11[6]	1.65±0.01[1]	0.79±0.006[1]

Table 6: Effect of different experimental diets on serum creatinine, albumin, total cholesterol, potassium and sodium levels

Groups	Days	Creatinine (mg/dl)	Albumin (g/dl)	Total Cholesterol (mg/dl)	Potassium (mEq/L)	Sodium (mEq/L)
G1	15	0.88±0.03^{a}	4.53±0.21^{a}	88.91±3.6^{a}	5.19±0.31^{a}	141.9±3.6^{ab}
	30	0.85±0.02^{1}	4.53±0.29^{1}	90.21±4.2^{1}	5.21±0.33^{1}	142.1±4.2^{1}
G2	15	1.29±0.03^{b}	5.73±0.21^{b}	121.80±6.7^{b}	6.12±0.42^{b}	152.8±5.3^{c}
	30	1.32±0.04^{2}	5.81±0.19^{2}	150.12±12.5^{2}	6.70±0.45^{2}	166.7±6.2^{2}
G3	15	1.01±0.01^{c}	5.23±0.17^{ce}	78.92±2.9^{ac}	6.01±0.39^{b}	151.7±5.9^{c}
	30	1.12±0.01^{3}	5.18±0.31^{3}	112.9±3.4^{3}	6.51±0.38^{2}	160.2±5.8^{3}
G4	15	0.99±0.003^{c}	5.20±0.29^{cd}	107.5±2.9^{b}	5.85±0.25^{b}	150.9±4.5^{cd}
	30	0.90±0.005^{4}	5.21±0.25^{3}	116.5±4.3^{3}	6.01±0.32^{3}	159.2±4.2^{3}
G5	15	0.79±0.004^{d}	5.01±0.21^{f}	91.21±3.9^{a}	5.32±0.25^{a}	147.6±3.7^{d}
	30	0.72±0.005^{5}	4.99±0.21^{3,4}	89.30±2.7^{1}	5.39±0.27^{4}	146.2±3.9^{4}
G6	15	1.06±0.06^{e}	5.16±0.22^{c}	76.51±2.5^{ad}	5.90±0.30^{b}	143.5±2.9^{a}
	30	0.79±0.002^{6}	4.82±0.32^{4}	79.15±4.6^{1}	5.91±0.33^{3}	142.6±3.7^{1,4}
G7	15	0.80±0.006^{d}	4.23±0.21^{de}	70.83±3.7^{cd}	5.40±0.12^{a}	138.6±2.1^{b}
	30	0.65±0.003^{7}	4.20±0.23^{5}	75.3±2.7^{1}	5.16±0.52^{1,4}	136.2±2.9^{5}

Table 7: Effect of different experimental diets on serum alkaline phosphates (ALP), lactate dehydrogenase (LDH), and glucose-6-phosphate dehydrogenase (G6PD) activities

Groups	Days	ALP (IU/L)	LDH (IU/L)	G6PD (U/min/mg protein)
G1	15	136.5±5.2^{a}	260.3±12.3^{a}	1.99±0.20^{a}
	30	136.2±4.9^{1}	261.1±11.2^{1}	1.98±0.19^{1}
G2	15	166.8±6.2^{b}	242.7±9.3^{b}	1.21±0.06^{b}
	30	180.2±6.9^{2}	233.5±8.3^{2}	1.18±0.05^{2}
G3	15	159.2±5.3^{c}	267.9±13.9^{ad}	1.39±0.08^{cd}
	30	179.0±7.3^{2}	263.0±10.1^{1}	1.42±0.07^{3}
G4	15	165.4±6.9^{b}	249.3±8.7^{b}	1.28±0.05^{bc}
	30	171.1±9.3^{3}	248.1±6.9^{3}	1.51±0.08^{3}
G5	15	140.7±3.2^{a}	272.3±11.3^{d}	1.75±0.11^{e}
	30	135.3±3.1^{1,4}	265.2±12.5^{1}	1.92±0.20^{1}
G6	15	152.2±5.1^{d}	260.9±9.7^{a}	1.45±0.08^{d}
	30	139.8±3.1^{1}	259.2±8.6^{1}	1.89±0.27^{1}
G7	15	128.2±2.2^{e}	266.4±9.7^{a}	1.93±0.25^{a}
	30	129.4±3.1^{4}	261.2±8.9^{1}	2.17±0.22^{4}

**Values are expressed as mean± S.D., n=10 -There was significant difference between means within the same column bearing different alphabetic superscripts on 15 days at p<0.01. - There was significant difference between means within the same column bearing different numerical superscripts on 30 days at p<0.01[For Table2. – Table 7.]*