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Beyond Metformin: Unveiling the Renoprotective Potential of Cholecalciferol and Taurine in Diabetic Kidney Disease

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Abstract: ***This research aims to determine whether cholecalciferol and taurine supplements may improve the efficacy of metformin in treating diabetic renal disease and reduce the risk of related complications. Materials and methods: the presented study was based on six distinct categories of rats, each consisting of six rats. Group I consisted of normal control rats (N); Group II contained diabetic control rats (D); Group III included diabetic rats treated orally with metformin. Group IV (Cho+M) consisted of diabetic rats that received a daily oral dosage of cholecalciferol plus metformin. Group V (TA+M) included diabetic rats treated with taurine in combination with a daily oral dose of metformin. Group VI involved diabetic rats that received a daily oral dosage of taurine, cholecalciferol, and metformin (Cho+TA+M). After six weeks, biological samples of blood and kidney tissues were gathered for biochemical, inflammatory markers levels and histological changes. Results: diabetic rats had elevated serum urea and interferon-gamma (INF-γ) levels, as well as reduced interleukin 10 (IL-10) levels in comparison to the normal control group. Additionally, several kidney histological alterations were noted in comparison to the normal control group. Combining cholecalciferol and taurine with metformin effectively improved all abnormal measurements. Cholecalciferol and taurine supplementation, along with metformin, may reduce diabetic kidney damage severity by leveraging their strong antioxidant, anti-inflammatory, and anti-apoptotic characteristics.***

Keywords: ***Type 2 diabetes mellitus (T2DM), Inflammation, Metformin, Cholecalciferol.***

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I. Introduction

One of the most serious microvascular consequences of T2DM, diabetic kidney disease (DKD) ultimately results in permanent renal damage and progresses to end-stage renal disease (ESRD) **(Hickey and Martin, 2018 ; Zhu *et al*., 2019 & Birnbaum *et al*., 2021)**. 20–40% of persons with DM have diabetic nephropathy (DN) at any one time **(Wang *et al*., 2014 &Lee *et al*., 2020)**.

Diabetic nephropathy (DN) pathophysiology is greatly affected by oxidative stress, inflammation, and hemodynamic changes. These changes may result from elevated levels of blood sugar (hyperglycemia) and increased lipids in the blood (hyperlipidemia). Prolonged hyperglycemia intensifies oxidative stress and contributes to the glycoxidation/peroxidation process. An excessive amount of reactive oxygen species within cells could exacerbate issues in DN's macrovascular and microvascular systems **(Mima, 2013)**. These excessively reactive free radicals promote kidney fibrosis and inflammation. Renal inflammation has a critical role in promoting the development and advancement of DN, according to **Matavelli *et al*. (2010); Navarro-González *et al*. (2011) & Bhatti *et al*. (2022).** IL-10 is a cytokine, had an essential anti-inflammatory effect in the prevention of autoimmune disease development. IL-10 dysregulation raises the likelihood of developing numerous autoimmune disorders and is linked to heightened immunopathology **(Iyer and Cheng, 2012)**. Moreover, the risk of diabetes is raised by differences in the IL-10 gene. IFN-γ is essential in the beginning of type 2 diabetes due to its pro-inflammatory properties **(Leung *et al*., 2018)**.

Additionally, podocytes of DN may undergo apoptosis because of Reactive oxygen species (ROS) produced by hyperglycemia **(Susztak *et al*., 2006)**, and apoptosis is a possible mechanism of cell loss in patients of T2DM **(Verzola *et al*., 2007)**. Therefore, DN may be considerably improved by medication with anti-inflammatory and antioxidant properties **(Lee *et al*., 2020)**.

Serum creatinine and urea tests are simple biomarkers to detect chronic renal failure whatever its cause (such as Diabetic nephropathy). In the case of diabetic nephropathy, vital indicators like urea and creatinine in serum are known to rise with high blood sugar in people with uncontrolled diabetes and are often linked to kidney damage. Blood levels of urea and creatinine help diagnose and early intervention to stop diabetic renal disease sooner **(Zimmet et al., 2001; Larsen and Kronenberg, 2011)**.

Among diabetics with type 2 diabetes, subclinical inflammation as well as the presence of almost all systemic inflammatory markers have been noted. The development of this illness is believed to be largely influenced by inflammation. T2DM, metabolic syndrome, and insulin resistance have all been linked to chronic systemic subclinical inflammation. Increased levels of inflammatory markers, such as inflammatory cytokines are present in the bloodstream during this systemic and subclinical inflammatory process **(Elimam *et al*., 2019)**.

Prior to starting metformin medication and on a regular basis while taking it, renal function must be evaluated, especially in older patients to correctly determine any substantial level of kidney impairment **(Bailey, 2005).** Metformin is a well-recognized medicine used to control diabetes and has shown efficacy in treating various diabetic complications **(Ravindran *et al*., 2017 & Lin *et al*., 2018)**.

Unfortunately, since it was expelled via the kidneys and had some renal toxicity, metformin has no direct role in improvement of diabetic nephropathy. Therefore, it is crucial to create innovative medications with minimal toxicity for the treatment of DN **(Ma *et al*., 2020).** Thus, taurine and cholecalciferol were used in this study because of their anti-inflammatory and antioxidant properties.

Cholecalciferol (Vitamin D3) is a steroid hormone and the only one that isn't made from cholesterol. Multiple activities are illustrated by VD3, which is involved in immunological and metabolic processes in addition to maintaining bone and mineral homeostasis. It has also been noticed that cholecalciferol has anti-inflammatory properties **(Leal *et al*., 2020)**.

Among the essential amino acids, taurine is one of the most important with sulfur (2-aminoethane-sulfonic acid) **(Maleki *et al*., 2020)**. In both animal models and people, taurine improves insulin sensitivity and secretion, acting as a preventative against the development of diabetes **(Ito *et al*., 2012 & Imae *et al*., 2014).** Taurine administration is advantageous and protective against tissue damage brought on by DM **(Ito *et al*., 2012).** This study seeks to determine if cholecalciferol and taurine supplements may improve the efficacy of metformin in treating renal damage resulting from diabetes mellitus and reduce the likelihood of related complications.

II. Materials and methods

Chemicals: STZ and taurine were acquired from Sigma-Aldrich in St. Louis, MO, and the Middle East Company. However, other medications like metformin and cholecalciferol were bought from a local pharmacy.

A rat model of diabetes was created experimentally as follows: A total of 75 male Sprague Dawley rats weighing between 200-250g were kept in the animal house at the Department of Zoology, Faculty of Science, Zagazig University. Diabetes was induced in rats by administering a dosage of 45 mg/kg of STZ by intraperitoneal injection in pH 4.5 citrate buffer, as described by **Qi et al. (2020)**. In order to address the drug-induced condition of low blood sugar, rats were administered STZ injections and then provided with a 20% fructose solution overnight. Rats whose fasting blood glucose levels more than 250 mg/dl were categorized as diabetic and as diabetic and participated in the research, whereas the remainder rats were excluded. The rats were kept in hygienic cages under controlled circumstances, with a food rich in fat and unrestricted access to water, following a 12-hour light and dark cycle. The ZU-IACUC Committee granted ethical approval for this investigation (Ethical sanction number: ZU-IACUC/ 2/ F/ 47/ 2022).

Grouping of the animals: Sixty rats in all were divided into six groups, each with 10 rats. Group I comprised healthy control rats, while Group II included rats with streptozotocin-induced diabetes (D). Group III received a 6-week treatment of oral metformin (M) at a dose of 250 mg/kg, following the regimen outlined by Subhasree et al. (2015). In Group IV (Cho+M), diabetic rats were given a daily oral dose of cholecalciferol (7500 IU/kg), based on **Poisbeau *et al.*'s (2019)** study, in addition to daily metformin (250 mg/kg) for 6 weeks. Group V (TA+M) involved diabetic rats treated with taurine at a dose of 100 mg/kg, following the protocol by Caletti et al. (2012), along with a daily oral dose of metformin (250 mg/kg b.wt) for 6 weeks. Group VI (diabetic rats) treated

with daily oral doses of taurine (100 mg/kg), cholecalciferol (7500 IU/kg), and metformin (250 mg/kg) for 6 weeks.

Body weight: The weight of the rats was determined during the six weeks of the experiment. The change in the body weight was calculated.

Following the designated therapeutic period, the rats underwent a period of fasting during the night. Both blood and kidney tissue were collected in containers kept at a low temperature for investigation of biochemical and inflammatory biomarkers, as well as for histological analysis.

Biochemical studies: Serum creatinine was measured enzymatically using Diamond kit according to **Burtis *et al.* (1999).** The Biomed kit was used to assess serum uric acid enzymatically, according to **Trinder (1969)**. Meanwhile, the Diamond kit was calorimetrically employed to estimate serum urea, according to **Young (2001).** IL-10 and INF-γ levels were estimated in the sample of rat's cell culture supernatants, plasma, and serum samples by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available kits [Rat IL-10 ELISA Kit (***ELR-IL10-1***), and Rat INF-γ ELISA kit (***ELR-IFNg-5***).

Statistical Analysis: The data were presented as the mean value ± the standard error for a sample size of 6. 'P' values below 0.05 were deemed significant. The statistical analysis was conducted using SPSS version 26 software, while GraphPad Prism 9.0 was used for creating graphics. Data analyses were conducted using one-way analysis for most variables, except for body weight data, which was analyzed using two-way analysis of variance (ANOVA).

III. Results

Body weight change: The data collected in Figure 1 and Table 1 indicated body weight changes in the normal and different experimental groups. The data showed that the diabetic group (D) began to show a significant decrease in body weight beginning from the 3rd week until the 6th week compared with the normal control group. On the other hand, the M group compared with the D group didn't show any significant difference in body weight. Also, the TA+M treated group did not show any significant change in body weight when compared to the D and M groups. Compared to the D and the M groups from the 3rd week until the 6th week the Cho+M treated group showed a significant increase in body weight. In comparison to the D and M groups, the administration of Cho, TA, and M showed a significant increase.

Table1: Body weight change in control and different experimental groups. Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. **$** significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

Weeks	Change in body weight (gm)

Groups	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week
Normal	5.80 ± 2.72	11 ± 2.95	16.4 ± 3.78	21.6 ± 4.62	26.6 ± 6.33	29.8 ± 5.15
D	-9 ± 2.17	-15.83 ± 2.57	-21.33 ± 2.89*	-26.83 ± 3.82*	-30.33 ± 4.65*	-35.67 ± 5.87*
M	-14 ± 3.17	-21.40 ± 5.63	-26.4 ± 6.15*	-32.8 ± 9.1*	-39.2 ± 10.37*	-44.8 ± 10.42*
Cho+M	-2 ± 3.48	-3.25 ± 4.72	7.75 ± 6.73#$	11.75 ± 4.91#$	17.75 ± 7.420#$	22.5 ± 7.71#$
TA+M	-4.67 ± 0.98	-12.17 ± 1.17	-14 ± 4.3*	-17 ± 4.9*	-20 ± 5.76*	-27.17 ± 9.1*
Cho+TA+M	-8 ± 1.40	-1.8 ± 3.32	4.8 ± 4.19#$	7.8 ± 5.77#$	8.6 ± 6.74#$	13.4 ± 8.3#$

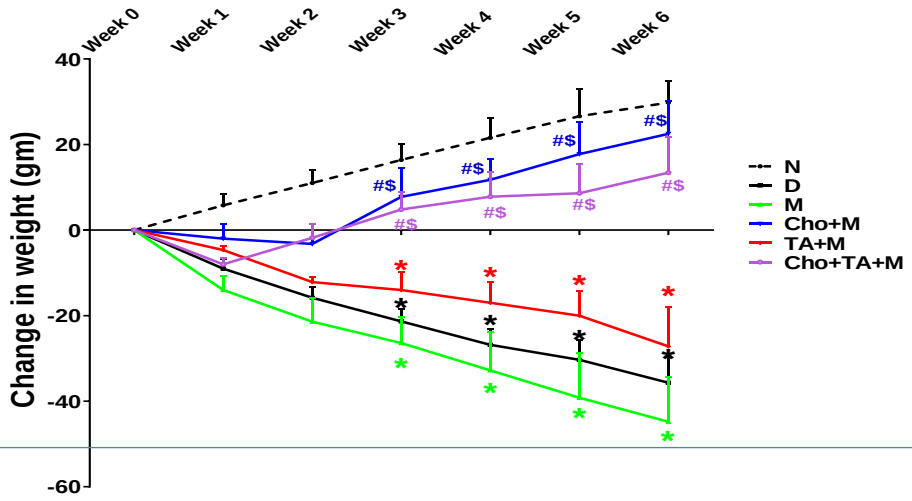


Figure 1: Body weight change in normal and different experimental groups. Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. $ significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

• Levels of serum creatinine, uric acid, and urea: As shown in Table 2 and Figures (2&3), The renal parameters, such as the serum creatinine and uric acid, were within the ordinary range in the D group and treated groups, except the Cho+TA+M treated group had a significant decrease in serum creatinine level compared to the D and the M groups. That was accompanied by an increased serum urea level in the D group compared to the normal group. On the other hand, the treated groups M and Cho+M had no significant change in serum urea level when compared with the D group. However, the Cho+TA+M-treated group illustrated a significant reduction in serum urea compared to the D and M groups.

Groups	Serum Creatinine (mg/dl)	Uric acid (mg/dl)	Serum urea (mg/dl)

Table 2: Serum creatinine, uric acid and blood urea levels in control and different experimental groups.

Normal	**0.75 ± 0.02**	**6.87 ± 0.12**	**38.67 ± 2.07**
D	**0.88 ± 0.03**	**7.13 ± 0.91**	**60.5 ± 3.39***
M	**0.91 ± 0.13**	**7.03 ± 0.38**	**53.33 ± 9.07**
Cho+M	**0.87 ±0.07**	**6.93 ± 0.55**	**50.17 ± 2.92**
TA+M	**0.73 ± 0.03**	**7.15 ± 0.53**	**39.67 ± 1.54#**
Cho+TA+M	**0.55 ± 0.03#$**	**6.62 ± 0.56**	**32.67 ± 2.36#$**

Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. **$** significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

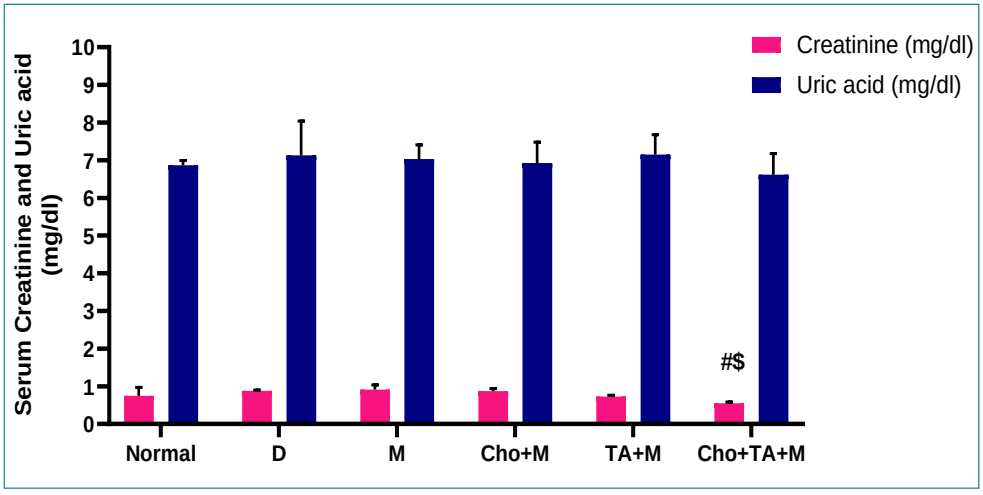


Figure 2: **Serum creatinine and uric acid levels in control and different experimental groups.** Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. $ significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M=

Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

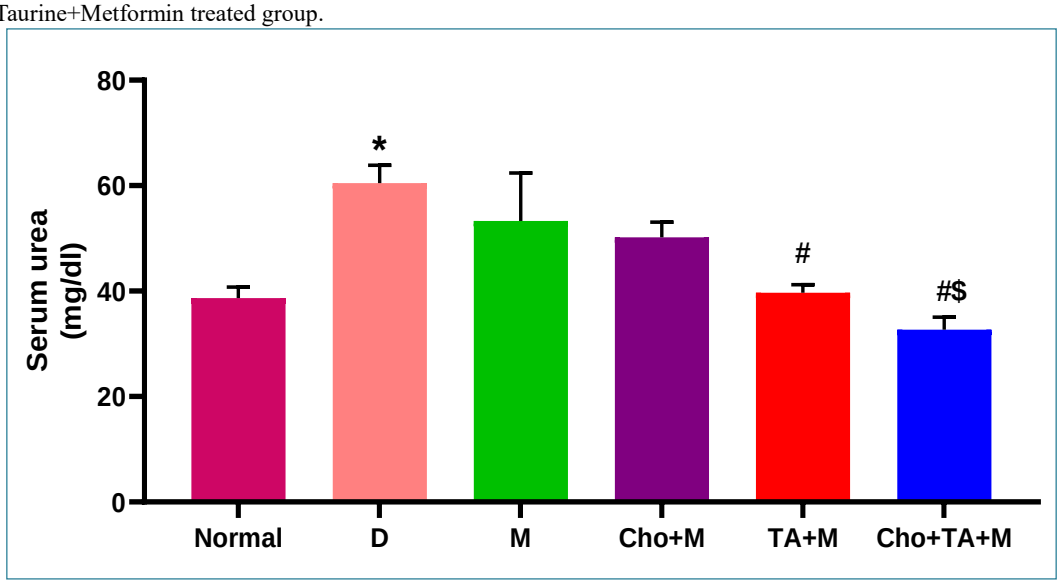


Figure 3: Serum urea level in control and different experimental groups. Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. $ significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

IL-10 and IFN-γ levels: Presented data in table 3 and figure 4 showed a significant increase in INF-γ and a significant decrease in IL-10 levels in the D group compared to normal group. The M- treated group did not show a significant difference in INF-γ and IL-10 levels when compared to the D group. But the Cho+TA+M treated group showed a significant decrease and a significant increase in INF-γ and IL-10 levels respectively when compared to the D and M groups.

Table 3: Levels of interleukin 10 (IL-10) and interferon-gamma (IFN-γ) in the serum of both control and various experimental groups were measured. Data stated as means ± SE (n=6). Values were significantly different at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. $ significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

Groups	IFN-Y (Pg / ml)	IL-10 (Pg / ml)
Normal	11.17 ± 0.94	29.33 ± 2.64
D	21.67 ± 1.92*	12.17 ± 1.53*
M	16.17 ± 1.14*	13.67 ± 0.88*
Cho+M	11.50 ± 1.17#$	32.00 ± 3.50#$
TA+M	13 ± 0.57#	21.50 ± 1.83#
Cho+TA+M	9.50 ± 0.76#$	37.50 ± 2.79#$

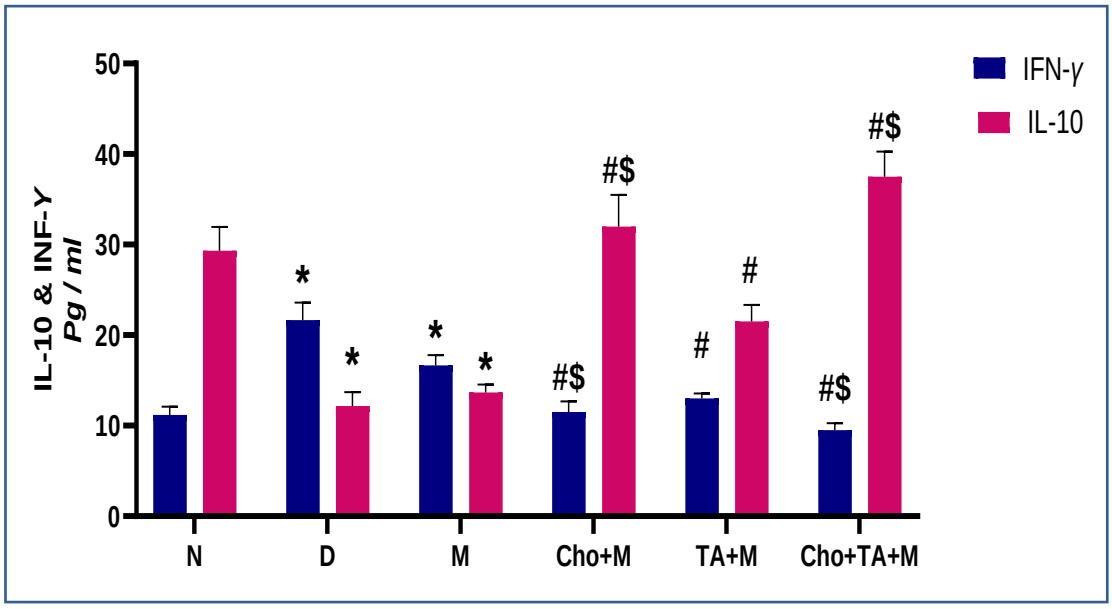
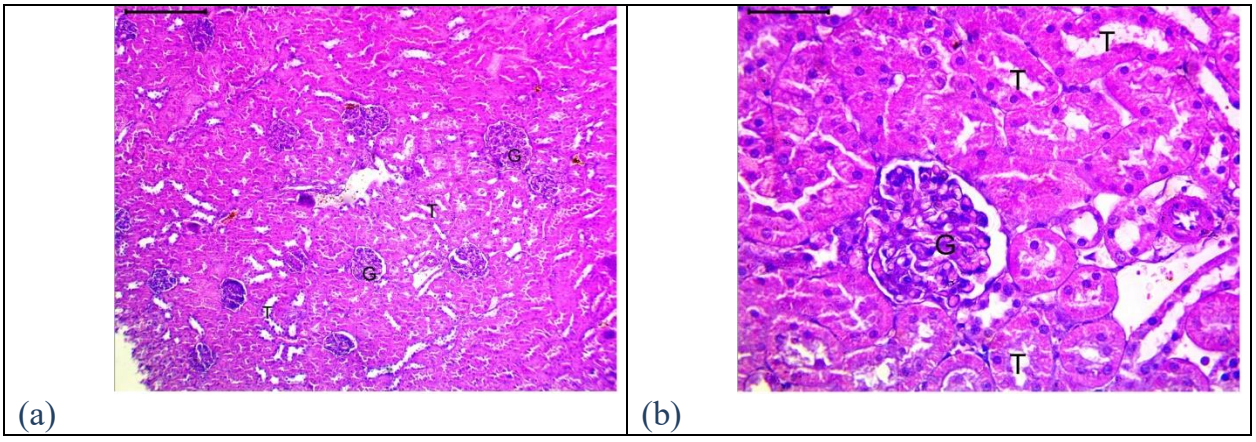
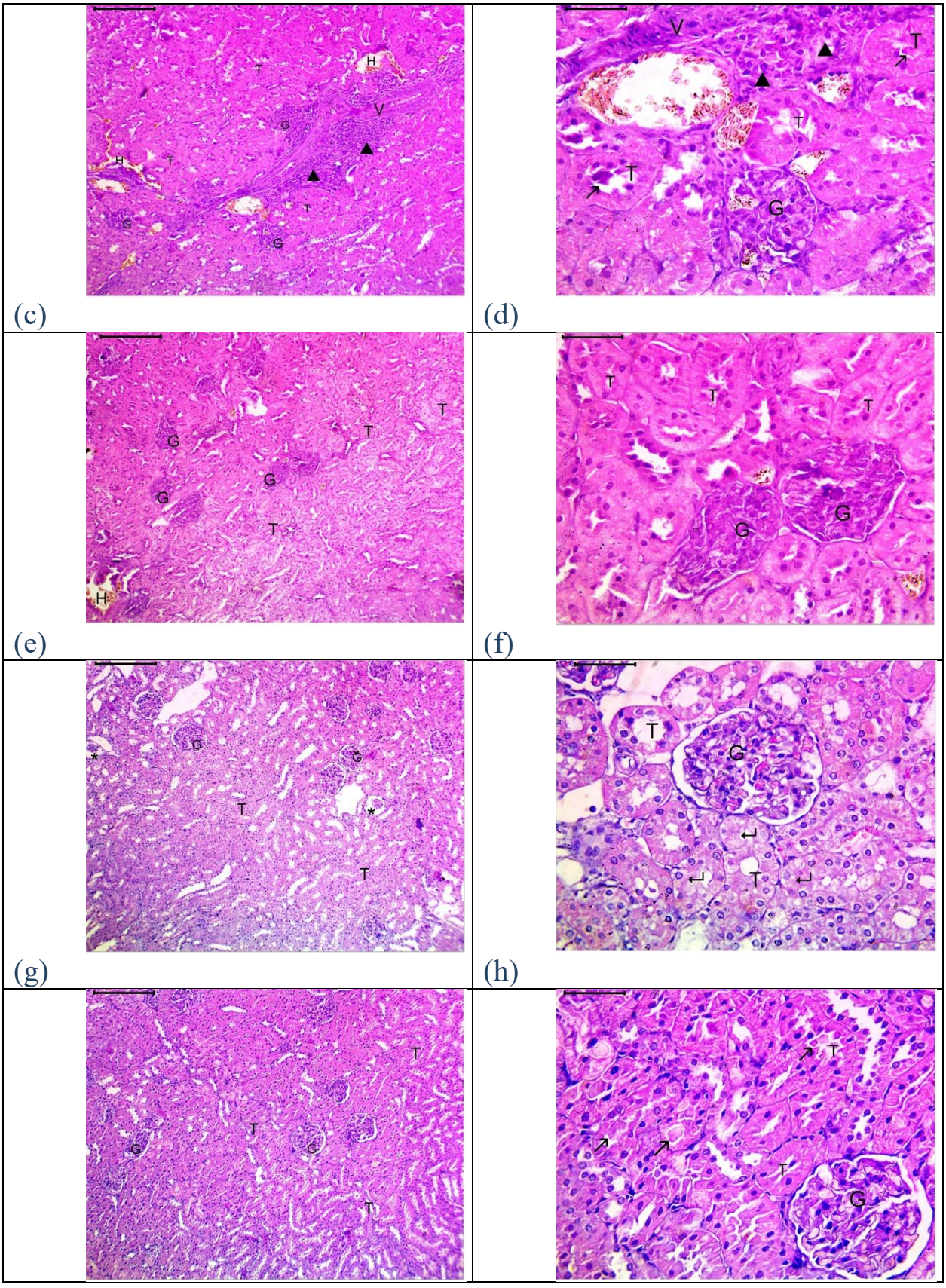


Figure 4: Levels of interleukin 10 (IL-10) and interferon -gamma (IFN-γ) in the serum of both control and various experimental groups were measured. Data stated as means ± SE (n=6). Values revealed a significant difference at $p < 0.05$. * Significant compared to the normal group. # significant compared to the D group. $ significant compared to the M group. D = diabetic group. M= Metformin treated group. Cho+M= Cholecalciferol + Metformin treated group. TA+M= Taurine+Metformin treated group. Cho+TA+M= Cholecalciferol+Taurine+Metformin treated group.

Histological observations

Figure 5 displays the findings of H&E staining for section of the kidney that were acquired through histological examination. (Figure 5 a, b) the healthy control group showed the normal structure of glomeruli. While the diabetic rats (D) showed marked inflammatory infiltrate and hemorrhage found in the interstitial tissue with markedly thickened blood vessel (Figure c, d). Also, it was also observed that the M-treated group had markedly narrowed tubular lumens and interstitial hemorrhages (Figure 5 e) as well as a narrowed tubular lumen with swelling of the epithelial tubular lining and sclerosed glomeruli with narrow Bowman's spaces, glomeruli, and tubules (Figure 5 f).Even though, the (Cho+M)- treated group showed normal structure of glomeruli, and tubules fibers (Figure 5 g) and showed normal structure of glomeruli, with hydropic degeneration of tubular epithelium, glomeruli, and tubules (Figure 5 h). At the same time, the TA+M- treated group showed narrowing of tubular lumen, glomeruli, and tubules (Figure 5 i) while Figure 5 j of the TA+M- treated group showed narrowing of tubular lumen with cast formation denoting the tubular degeneration, glomeruli, and tubules. Nevertheless, glomeruli and tubules of Figure 5 k and Figure 5 l (Cho+TA+M- treated group) exhibited normal structure as well, including an intact Bowman space.




(c)
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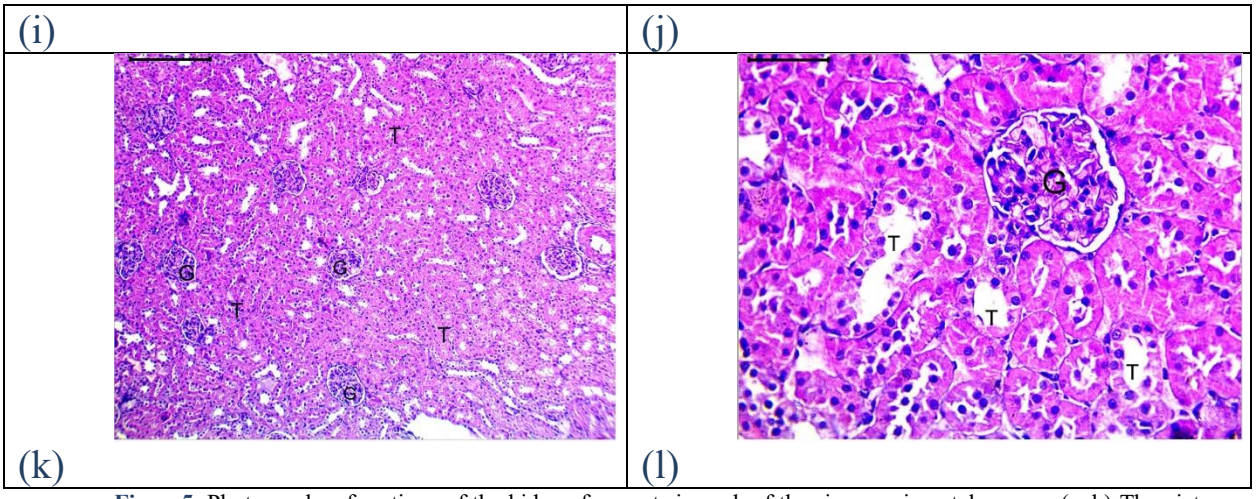

Figure5: Photographs of sections of the kidney from rats in each of the six experimental groups. (a, b) The pictures of the normal group showed that the glomeruli (G) and tubules (T) were normal in structure. (b) is a higher magnification of (a). (c) Photograph of the diabetic group (D) illustrated marked inflammatory infiltrate (arrowheads) and hemorrhage (H) found in the interstitial tissue with markedly thickened blood vessel (V). glomeruli (G), and tubules (T) while (d) Photograph of the diabetic group (D) showed glomerular sclerosis with narrow Bowman's space and tubular casts (arrows), marked inflammatory infiltrate (arrowheads) and hemorrhage (H) are found in the interstitial tissue with markedly thickened blood vessel (V). glomeruli (G), and tubules (T). (d) is a higher magnification of (c). (e, f) Photographs of the M-treated group showed marked narrowing of tubular lumens, swelling of epithelial tubular lining, sclerosed glomeruli with narrow Bowman's space and interstitial hemorrhage (H). (f) a higher magnification of (e). (g, h) Photographs of the Cho+M- treated group showed that normal structure of glomeruli (G), and tubules (T) with hydropic degeneration (left-sided arrows) of tubular epithelium (noted by swollen lining with faint staining). (h) is a higher magnification of (g). (i) Photograph of the TA+M- treated group illustrated narrowing of tubular lumen, glomeruli (G), and tubules (T). (j) Photograph of the TA+M- treated group showed narrowing of tubular lumen with cast formation denoting the tubular degeneration (arrows), glomeruli (G), and tubules (T). (j) is a higher magnification of (i). (k, l) As shown in the images of the Cho+TA+M-treated group, the glomeruli (G) and tubules (T) had normal structure. (l) is a higher magnification of (k). Scale bars of photographs (a), (c), (e), (g), (i), and (k) = 100 *um* and scale bars of photographs (b), (d), (f), (h), (j) and (l) = 40*u*m.

IV. Discussion

Meanwhile, a typical sign of untreated, decompensated diabetes after induction of STZ is weight loss **(Olokoba *et al*., 2012)**. Our results revealed that the diabetic group began to show a distinguished decline in body weight beginning from the 3rd week until the 6th week compared with the normal group. This agrees with **Sturza *et al*. (1982); Zhang *et al*. (2008); Hikmah *et al*. (2015); Tian *et al*. (2016) and Santos *et al*. (2021)**. The cause of weight loss is that the body develops routes to gain energy by enhancing gluconeogenesis, lipolysis, and ketone synthesis. This is done because tissues cannot absorb glucose due to metabolic dysfunction **(Ferreira *et al*., 2011 & Soliman, 2016). Sadri *et al*. (2017)** also reported that this loss could be the result of catabolism or the breakdown of proteins and lipids. Therefore, enhanced catabolic processes led to a loss of muscle mass, which is a major factor in the weight loss of diabetic rats**.** In contrast, the studies carried out by **Bowonsomsarit *et al*. (2021); Zhang *et al*. (2021) & Moharir *et al*., (2022). Bowonsomsarit *et al*. (2021) & Moharir *et al*., (2022)** illustrated that there was a significant gain in body weight of the STZ diabetic rats compared to the control whereas **Zhang *et al*. (2021)** explained that there was no significant change in the body weight between the STZ 45 and control groups.

On the other hand, from the 3rd week until the 6th week the synergistic administration of cholecalciferol + metformin and cholecalciferol + taurine + metformin to diabetic rats minimizes body weight loss. This showed this combination treatment plan may be able to stop several metabolic abnormalities linked to muscle atrophy and the loss of adipose tissues.

Chronic kidney disease (CKD) is becoming more common **(Abboud and Henrich,2010 & Slee, 2012)** and its metabolic and hormonal consequences have been the topic of several investigations. Type 2 diabetes (T2D), a primary contributor to end-stage renal disease (ESRD), poses a substantial and increasing health concern **(Fox *et al*., 2015 & Dion *et al*., 2017).** The markers used to diagnose renal function include serum creatinine and urea **(Griffin *et al*., 2008 & Singh *et al*., 2014).** Anjaneyulu et al.'s 2004 study discovered that rising serum creatinine and urea levels in diabetic rats signify increasing kidney impairment. The data obtained showed that the serum creatinine level did not exhibit a significant change in the STZ diabetic rats when compared to the control group. These

findings align with **Nabil *et al.* (2015)** who observed that the serum creatinine remained normal**.** Also, **Bamanikar *et al.* (2016)** observed slow and minimal rise in serum creatinine in his samples with hyperglycemia. However, **Dhein *et al.* (2000)** noted elevated serum creatinine in diabetic rats three months after diabetes induction, whereas our study was conducted over a shorter time. It also increases in the case of chronic renal failure, and this was not achieved in our study due to the short duration. It is an insensitive biomarker of early renal impairment because normal serum creatinine concentration may be achieved even when the GFR has significantly decreased **(Mann, 1999 & Nabil *et al.*, 2015).** This disagrees with **Bamanikar *et al.* (2016)** who reported that there was no correlation between the length and intensity of diabetes and serum creatinine levels.

In contrast, the combination treatment with cholecalciferol, taurine, and metformin reduced serum creatinine level of rats as compared to the diabetic and M-treated groups. This disagrees with **Wang *et al.* (2019)** who stated that no evidence Showing that cholecalciferol supplementation lowers serum creatinine level. However, the results from the current study align with the findings of **Mozaffari and Schaffer, (2001) & Baliou *et al.* (2021).**

Conversely, serum urea level increased in the D group as compared to the normal group in this presented study. This study is in harmony with **Anjaneyulu and Chopra, (2004); Pareek *et al.* (2009); Shrestha *et al.* (2008); Singh *et al.* (2014) & Bamanikar *et al.* (2016)** who demonstrated that inadequate management of blood sugar levels may lead to elevated serum urea levels, hence enhancing the possibility of the patient getting diabetic nephropathy, progressive renal impairment may be due to several factors, including hyperglycemia. An elevation in the amount of urea is seen when the kidneys are damaged or mishandled and an increase in urea level in parallel with a rise in blood sugar level amply demonstrates that rising blood sugar levels harm kidneys. While **Pareek *et al.* (2009)** illustrated that the cause of elevated urea production is due to increased liver protein and plasma catabolism.

Regarding inflammatory markers, diabetic rats exhibited a notably significant rise in IFN-γ levels and a markedly significant reduction in IL-10 levels compared to the normal rats. On the same path, **Lv *et al.* (2021) & Halimi *et al.* (2022)** confirmed that there was a notable rise in IFN-γ level and a notable fall in IL-10 level in diabetic rats. This salient finding of the present study comes in harmony with **Wensveen *et al.* (2015); Saad *et al.* (2016) & McLaughlin *et al.* (2017**), who reported that individuals with Type 2 Diabetes (T2DM) may experience inflammation. However, following a combination therapy of cholecalciferol, taurine, and metformin, there was a notable increase in interleukin-10 (IL-10), a recognized anti-inflammatory cytokine. The heightened presence of IL-10 in treated diabetic rats suggests a complex interplay with its pro-inflammatory counterpart, interferon-gamma (IFN-γ). This elevated IL-10 expression may indicate its role as a compensatory and counter-regulatory molecule by curbing pro-inflammatory cytokine production and suppressing the activity of pro-inflammatory natural killer cells, T-helper 1 cells, and macrophages to limit inflammatory damage. **(Moore *et al.*, 2001 & Botha-Scheepers *et al.*, 2008).**

The histological investigations for Ht & E of the kidney tissue of the diabetic rats confirmed that glomerular sclerosis with narrow Bowman's space and tubular casts. Marked inflammatory infiltrate and hemorrhage are found in the interstitial tissue with markedly thickened blood vessel. These results of the present study are corroborated by **Nabil *et al.* (2015); M and MM, 2018 & Ebrahim *et al.* (2019).**

Meanwhile, the histopathological examination of Ht & E of the kidney tissue of the Cho+TA+M- treated group illustrated that the structure of glomeruli, and tubules was normal and this refers to using the treatment combination: cholecalciferol, taurine, and metformin because of their protective roles as antifibrotic and anti-inflammatory substances. This finding is in accordance with **Santoro *et al.* (2015); Gembillo *et al.* (2019); Baliou *et al.* (2021) & Kaur *et al.* (2021).**

V. Conclusion

The current study can prove the effectiveness of cholecalciferol and taurine supplements along with antidiabetic drug (metformin). As diabetic rats exhibited a notable rise in urea levels, proinflammatory cytokines, and a significant drop in anti-inflammatory cytokines. At the same time the histological changes confirmed the presence of inflammation. After 6 weeks of treatment, metformin monotherapy could not completely treat diabetic rats. So, using combination therapy of cholecalciferol and taurine with metformin can prevent or at least stop the progression of diabetic nephropathy.

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