**International Journal of Zoology and Applied Biosciences** **ISSN: 2455-9571**
**Volume 10, Issue 6, pp: 52-64, 2025**
**https://doi.org/10.55126/ijzab.2025.v10.i06.007** **http://www.ijzab.com**

**Research Article**

# *GNETUM GNEMON* LEAF EXTRACT AS A POTENTIAL THERAPEUTIC AGENT FOR NEPHROTOXICITY AGAINST DALTON'S LYMPHOMA ASCITES (DLA)-INDUCED MICE

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**Article History:** Received 26th August 2025; Accepted 20th October 2025; Published 1st November 2025

## ABSTRACT

Melinjo (*Gnetum gnemon*) is traditionally known for its anti-inflammatory, antioxidant, and anticancer properties. However, its role in preventing cancer-related kidney damage is underexplored. This study examined the protective effects of *Gnetum gnemon* methanolic leaf extract (GGME) on DLA-induced (Dalton's lymphoma ascites) nephrotoxicity in Swiss albino mice. Two experiments were conducted using male albino mice (n = 5/group). In the acute study, Group I received PBS (control) and Group II received GGME (2000 mg/kg) for 14 days. In the chronic study, Group I was control, Group II -DLA-induced, Group III- DLA + cisplatin (2 mg/kg), Group IV- DLA + GGME (200 mg/kg), and Group V -DLA + GGME (400 mg/kg). DLA was induced by intraperitoneal injection of $1 \times 10^6$ live DLA cells per mice, and the experiment continued for 21 days. Kidney function biomarkers (BUN, creatinine, uric acid), key electrolytes ($Na^+$, $K^+$, $Ca^{2+}$), oxidative stress markers, and tissue histo-morphometry were evaluated. GGME showed no signs of toxicity at doses up to 2000 mg/kg. Mice in the group II (DLA-induced) exhibited significant nephrotoxicity, characterized by elevated BUN, creatinine, uric acid, altered electrolyte levels, increased oxidative stress, and notable renal tissue degeneration. GGME treatment, especially at 400 mg/kg, improved biochemical markers, restored antioxidant enzymes, and reduced MDA levels. Histology showed preserved kidney structure and reduced glomerular damage, with improvements in glomerular number, size, and capsular space. GGME exhibited dose-dependent nephroprotection in DLA-induced nephrotoxicity, likely via its antioxidant and anti-inflammatory actions, suggesting its potential as a complementary therapy for cancer-related kidney damage.

**Keywords:** Dalton's lymphoma, *Gnetum gnemon*, Kidney, Nephrotoxicity, Creatinine.

## INTRODUCTION

Kidneys are vital homeostatic regulators that perform three key functions: blood filtration and waste excretion, maintenance of precise electrolyte and fluid balance, and endocrine regulation of blood pressure (via the renin-angiotensin system) and erythropoiesis (via erythropoietin) (Temirova & Asadova, 2025). They are highly susceptible to damage from oxidative stress, inflammation, ischemia, diabetes, and nephrotoxic drugs-factors that often combine to cause progressive renal dysfunction or even complete failure (Jivishov *et al*., 2020). Nephrotoxicity refers to acute or chronic impairment of kidney function caused by exposure toxic substances including drugs and other chemicals. Various forms of nephrotoxicity exist, and some drugs may impair renal function through multiple mechanisms. Nephrotoxins are substances that exhibit nephrotoxic effect (Al-Naimi *et al*., 2019). Notably, nephrotoxicity is a common complication associated with various cancers and their treatments. Dalton's lymphoma ascites (DLA), a transplantable tumor model, is known to induce significant renal damage in experimental animals making it a suitable model for studying nephrotoxicity. The search for natural compounds with nephroprotective properties has led to the exploration of various medicinal plants. Natural products from plants are becoming increasingly popular due to their ability to reduce inflammation, combat oxidative stress, and protect renal function (Sun *et al*., 2024). For centuries, natural products

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have traditionally served as a key reservoir for bioactive molecules, offering a wide diversity of compounds capable of targeting several cellular pathways (Bustos-rangel *et al*., 2023). *Gnetum gnemon*, commonly known as Melinjo belongs to the family Gnetaceae, found in the tropical and mountainous forests of Southeast Asia and the Western Pacific region. This evergreen tree plays a vital role in enhancing nutritional intake in areas with limited access to protein-rich foods (Rahman *et al*., 2024).

Traditionally, this plant is recognized for its antioxidant, anti-inflammatory, and anticancer properties (Chatatikun *et al*., 2024). Preclinical studies have demonstrated that *G. gnemon* seeds are a potent source of antioxidant. Furthermore, extracts of *G. gnemon* have exhibit anticancer activity against the several cancer cell lines. Especially, the seed extract of *G.gnemon* showed significant cytotoxic effects against MCF-7 and HeLa cell lines (Sukohar & Ramdini, 2023). *Gnetum gnemon* seed extract and its active compound, gnetin C, a dimer of resveratrol, exhibited strong antitumoral activity in the proliferation of a wide variety of human and murine cancer cell lines through caspase-dependent and caspase-independent apoptotic pathways. *Invivo* evidence shows that treatment with Melinjo seed extract (MSE) by oral administration is capable of suppressing tumor growth, angiogenesis, and metastasis, thus giving this extract strong potential as a plant-based anticancer agent (Narayanan *et al*., 2015a). Moreover, MSE powder has been considered non-toxic in acute and sub-chronic oral toxicity studies as well as in a micronucleus test, thereby supporting its application as a safe therapeutic candidate and possible use in food applications (Tatefuji *et al*., 2014). The present study aims to evaluate the potential nephroprotective effects of *Gnetum gnemon* in Swiss albino mice subjected to Dalton's lymphoma ascites-induced nephrotoxicity. Addressing this question fills a critical gap in current research, as no studies to date have explored whether *G. gnemon* can mitigate kidney damage specifically associated with lymphoma. This is significant because cancer-related renal injury often arises through distinct mechanisms compared to drug-induced nephrotoxicity. The findings could contribute to the growing body of evidence supporting plant-based therapeutic strategies for managing cancer-associated kidney damage.

## MATERIALS AND METHODS

### Animals

Male Swiss albino mice, weighing around 25-30 g, were acclimatized to the laboratory condition for fortnight. They were housed in groups of five in metal cages under clean conditions, with a controlled temperature of 22 ± 2°C, relative humidity between 50–60%, and a 12-hour light/dark cycle. All experiments were conducted during the light phase, following the guidelines of the Institutional Animal Ethics Committee. The experimental mice were identified by labeling their tails and were given standard rodent food and water *ad libitum*. The study procedures were reviewed and approved by the ethical committee at Cotton University (Reg No. 15/IAEC/CU/05/01/2021).

### Nephrotoxicity Induction of Dalton's Lymphoma in mice

Dalton's lymphoma cells were maintained in the ascitic form in Swiss albino mice and injected intraperitoneally with $1x10^6$ viable tumor cells per animal to induce the tumor in experimental animals. All mice groups received DLA induction except control group.

### Preparation of leaf extract

The *Gnetum gnemon* plant (family: Gnetaceae) was obtained from Chala Village, Charaideo district, in April 2021. The Botany Department of Guwahati University, Assam, India, taxonomically identified the plant material and created a herbarium for future reference. The voucher specimen (No. GUBH20508) was preserved in our laboratory. A total of 50 g of *G. gnemon* leaves were ground and transferred into a 500 mL conical flask containing 300 mL of methanol, and the mixture was macerated for three days. The extraction was periodically sieved through a muslin cloth at 24-hour intervals. To protect light-sensitive compounds, the amber conical flask was used. After extraction, the mixture was filtered using filter paper, and methanol was removed from the liquid using a rotary evaporator under reduced pressure. The remaining methanol was dried in an oven at a temperature of 40°C or below for at least 48 hours. Finally, the dried extract was stored in an amber glass bottle at 4°C (Zeghoud *et al*., 2021).

### Experiments

### Acute oral toxicity

The acute toxicity of the methanolic extract of GG (GGME) was assessed following OECD guideline number 423(OECD, 2001), using male Swiss albino mice divided into two groups (n=5) after a 14-day acclimatization period. Group I received the vehicle control (PBS), while Group II was exposed to GGME (2000 mg/kg). Mice were fed overnight before receiving the oral drug/vehicle treatment. The animals were kept under close observation for the first hour, monitored frequently for the next four hours, and thereafter observed once every 24 hours for a period of 14 days. Throughout the study, clinical observations were recorded to assess mortality, behavioral changes, and any other abnormalities.

### Determination of nephrotoxicity

The animals were divided into five groups (n=5): Group I (Control, received normal saline), Group II (Dalton's lymphoma ascites-induced mice only and acted tumor control), Group III (Dalton's lymphoma ascites-induced mice treated with cisplatin of 2mg/kg BW and treated as positive control), Group IV (Dalton’s lymphoma ascites-

induced mice treated with *GG* extract of 200mg/kg BW), Group V (Dalton's lymphoma ascites-induced mice treated with *GG* extract of 400mg/kg BW). The treatment was administered orally for 21 days following Dalton's lymphoma ascites induction(Guo *et al*., 2022).

**Sample Collection**

After the successful completion of each treatment period, the experimental mice were deprived of food overnight while being allowed free access to water. On the following day, the animals were anesthetized to minimize pain and stress during the procedure. Under anesthesia, approximately 2–2.5 mL of blood was collected from each mouse by cardiac puncture using a sterile syringe. Immediately after blood collection, the mice were humanely euthanized by cervical dislocation in accordance with standard ethical guidelines for animal experimentation (Dubiwak *et al*., 2021; Leta *et al*., 2021). All the blood samples were collected at the end of the experiment. Kidney tissues were sectioned at thickness of 4–5 µm for precise and thorough analysis (Badar *et al*., 2021).

**Determination of body weight**

The body weight of each mouse was recorded using a digital balance with a sensitivity of 0.001 g. The measurements were taken on days 0, 7, 14 and 21 of the experimental period. The recorded values were expressed as the mean ± standard error of the mean (SEM) following the method described by (Tesfa *et al*., 2025).

**Determination of relative kidney weight**

Percentage of relative kidney weight of each mice was determined based on equation (Dubiwak *et al*., 2022) and the measurement was recorded at the end of the experiment.

$$\text{Relative kidney weight} = \frac{\text{Kidney weight(g)}}{\text{Body weight (g)}} \text{x} 100$$

**Biochemical parameters**

Briefly, the blood samples were first allowed to clot at 4 °C for 2 hours, after which serum was obtained by centrifuging the blood samples at 3,000 rpm for 10 minutes. The serum was frozen and kept at a temperature of -80°C until utilized for biochemical tests. All biochemical markers (BUN, creatinine and uric acid) were measured using the COBAS INTEGRA 800 Autoanalyzer (Roche Diagnostics, Germany). BUN levels were estimated using urease method, creatinine was determined by the method of Jaffes reaction, and the uric acid level was measured with uricase method.

**Assessment of serum renal electrolytes parameters**

The estimation of serum electrolytes, including sodium ($Na^+$), magnesium ($Mg^{2+}$), and calcium ($Ca^{2+}$), was carried out using thawed serum samples stored at -20 °C. The samples were brought to room temperature and centrifuged at 3000 rpm for 5 min to ensure clarity before analysis. Electrolyte analysis was measured using the VITROS 5600 automated biochemistry analyzer, relying on ion-selective electrode methodology.

**Oxidative stress markers**

Initially, the kidneys were blended with ice-cold normal saline using a homogenizer. The obtained homogenates were subsequently centrifuged at 800 × g for 5 minutes at 4 °C to collect nuclear fragments. The supernatant obtained was subsequently centrifuged again for 15 minutes at 5000 × g at 4 °C to yield the post mitochondrial supernatant (Govindappa *et al*., 2019). The activities of renal enzymatic antioxidants (SOD) and (CAT) were assessed using the technique as described by Rizk *et al* (2023), while the MDA and GSH activity was evaluated through the method of Abouzed *et al* (2021).

**Histopathological analysis**

After sacrifice, kidney tissues were collected, fixed in 10% formalin, and processed for histopathological investigation. Kidney sections were stained using double staining techniques of hematoxylin and eosin and examined under a light microscope (Leta *et al*., 2021). The samples were collected at the end of the experiment.

**Morphometric analysis**

Morphometric analysis of the kidney was conducted by using image software Motic Images Plus 2.0. Firstly, prepared sections were captured at a magnification of 40x for detailed analysis of glomerular number, capsular space area, and glomerular area.

**Statistical analysis**

The sample size per groups was calculated using power 80% with P value of < 0.05 using Lamorte's Power calculations and was found to be n=5. The data are presented as mean ± SEM and were tested for normality using the Shapiro–Wilk test. Two-way ANOVA was performed taking treatment and days as the two factors. When ANOVA indicated a significant difference, Bonferroni post hoc mean comparison tests were conducted. One-way ANOVA was used when only a single factor was involved. Regression analysis was also performed in the data. The effect sizes ($\eta^2$) were also calculated. A probability level of $p < 0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

The oral acute toxicity experiment revealed that administration of *G. gnemon* methanolic extract (GGME) at a dose of 2000 mg/kg body weight did not produce any significant toxic effects. During the 14-day observation period, the treated mice did not exhibit mortality or abnormal clinical signs. Behavioral assessments and neurological observations indicated the absence of central nervous system (CNS) disturbances such as tremors, convulsions, excessive salivation, abnormal respiration, or

alterations in pupil size. Similarly, no signs of aggressiveness, stereotypic repetitive behaviors, sedation, lethargy, or coma were observed. These findings suggest that GGME was well tolerated at the tested dose, with no evidence of acute toxicity under the study conditions. Further, there were no significant changes in the body weight as compared to control. Moreover, all of the GGME administered mice remained alive. The data thus obtained from these tests indicated that the $LD_{50}$ of GGME leaf was greater than 2000 mg/kg body weight. This led us to choose the doses of 200 mg/kg and 400 mg/kg of GGME leaf extract for the present chronic toxicity investigation.

A significant and time-dependent decrease in the body weight of mice was observed among the treatment groups (Days: $F_{3,\ 80} = 3.06$, $p = 0.0327$, $\eta^2 = 0.014$; Body weight: $F_{4,\ 80} = 112.1$, $p < 0.0001$, $\eta^2 = 0.727$; Interaction: $F_{12,\ 80} = 6.56$, $p < 0.0001$, $\eta^2 = 0.127$; Two-way ANOVA; (Figure 1 a). Group I (control) maintained a constant body weight throughout the exposure period. However, Group II (DLA-induced mice) showed a significant increase in body weight ($p < 0.0001$) on days 7, 14, and 21, due to tumor growth and ascitic fluid accumulation. In contrast, Group III (DLA + cisplatin) exhibited significant body weight loss ($p < 0.0001$) on days 7, 14, and 21, suggesting both tumor growth inhibition and potential drug-induced toxicity. Furthermore, Group IV (DLA + extract, 200 mg/kg BW) showed a slight weight loss, while Group V (DLA + extract, 400 mg/kg BW) exhibited a significant reduction in body weight ($p < 0.0001$) on days 7, 14, and 21 compared to the DLA control group. This indicates dose-dependent antitumor activity of the extract, which appeared to be less toxic than cisplatin. Regression analysis revealed no significant effect on body weight at low doses (200mg/kg; $r^2 = 0.0806$, $p = 0.2249$), whereas at high dose (400mg/kg; $r^2 = 0.2678$ and $p = 0.0194$) indicating a significant reduction in body weight in a dose dependent manner (Figure 1 b).

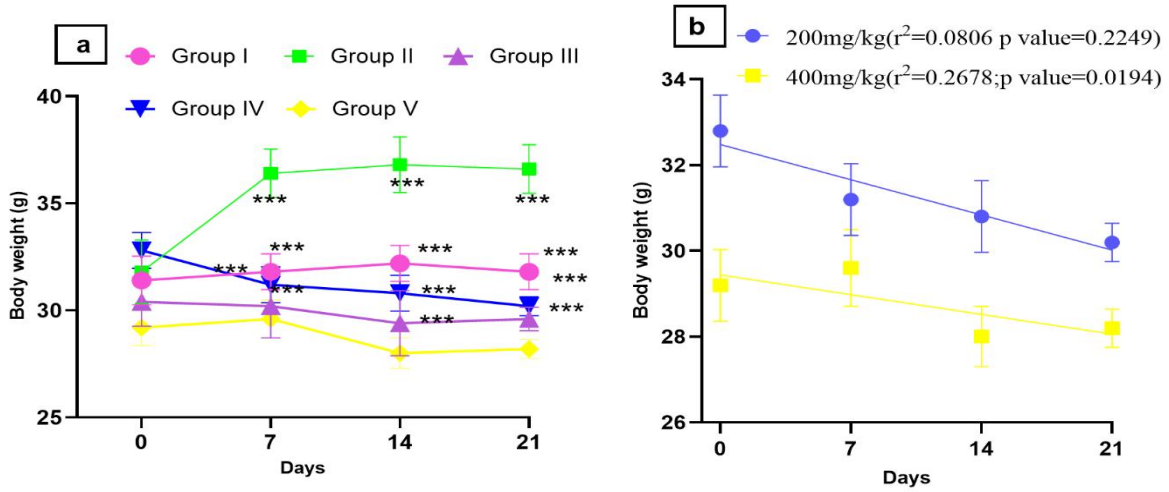


**Figure 1.** (a) Body weight changes in Dalton's lymphoma (DLA)-induced mice treated with *Gnetum gnemon* extract across all groups compared to control. (b) Regression analysis showing the effect of treatment on body weight in mice during nephrotoxicity.

A significant ($p$ value <0.0001) difference in relative kidney weight was observed in both extract-treated groups and the cisplatin-treated group compared to the tumor control group, indicating mitigation of tumor-related damage and partial restoration toward normal levels ($F_{4,\ 20} = 49.86$, $p < 0.0001$, $\eta^2 = 0.9088$; one-way ANOVA;( Figure 2). The control group (Group I) exhibited normal kidney weight relative to body weight, reflecting healthy kidney function. In contrast, the tumor-induced group (Group II) showed a significant decrease ($p < 0.0001$) in relative kidney weight, likely due to tumor-associated stress and physiological burden. Group III (cisplatin-treated) showed relative kidney weight comparable to that of the control group (Group I) and significantly higher ($p < 0.0001$) than the tumor-induced group (Group II), suggesting a protective or restorative effect. No significant difference ($p = 0.4050$) was observed between the lower-dose extract group (Group IV, 200 mg/kg) and the tumor control group (Group II), indicating limited efficacy at this dose. However, the high-dose extract group (Group V, 400 mg/kg) exhibited significantly higher relative kidney weight ($p < 0.0001$) compared to Group II, and similar values to the control group (Group I), suggesting that the extract at this dose helped restore kidney weight without causing much toxicity. Moreover, the 400 mg/kg treatment (Group V) demonstrated a significantly better kidney

weight profile ($p < 0.0001$) than the tumor-induced group (Group II), supporting its potential nephroprotective effect.

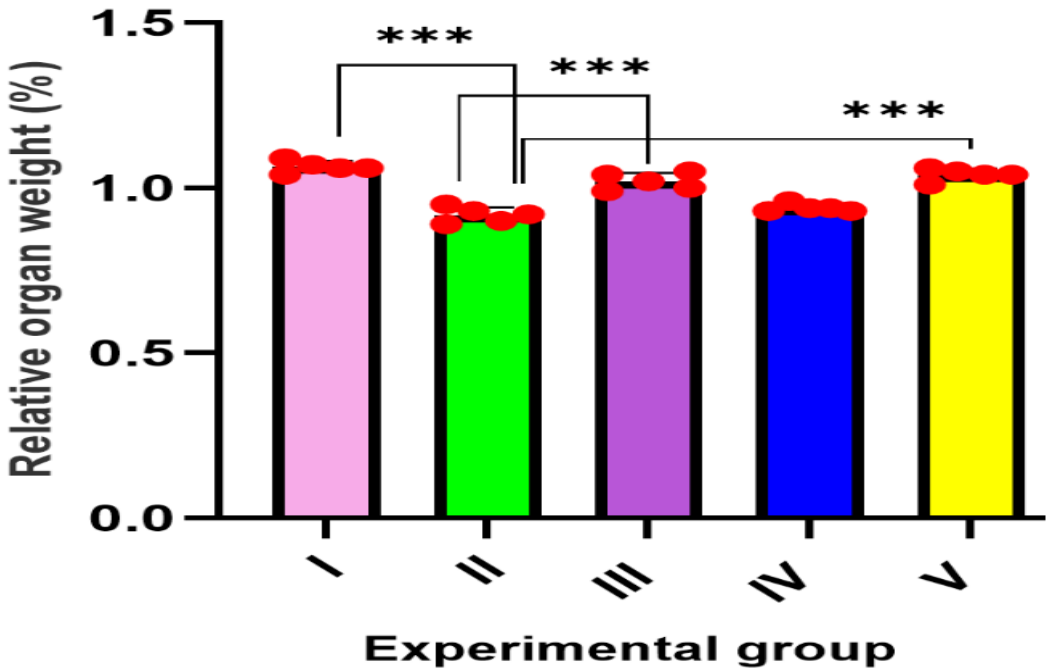


**Figure 2.** Effect of *Gnetum gnemon* extract on relative kidney weight in normal and DLA-induced mice.

Administration of GG extract at different doses significantly reduced serum BUN ($F_{4, 20} = 73.41$, $p < 0.0001$, $\eta^2 = 0.9361$; one-way ANOVA, (Figure 3a), creatinine ($F_{4, 20} = 147$, $p < 0.0001$, $\eta^2 = 0.9672$; one-way ANOVA, (Figure 3b), and uric acid levels ($F_{4, 20} = 107.4$, $p < 0.0001$, $\eta^2 = 0.9552$; one-way ANOVA, (Figure 3c) in lymphoma-bearing mice compared to the tumor control group (Group II). Treatment with the 200 mg/kg dose (Group IV) resulted in a highly significant decrease ($p < 0.0001$) in BUN, serum creatinine, and uric acid levels relative to the untreated tumor group (Group II), indicating protective effects on kidney function. A more pronounced effect ($p < 0.0001$) was observed with 200mg /kg and 400 mg/kg dose (Group V), where the levels of BUN, and uric acid were significantly lower, while creatinine levels are slightly elevated, as compared to control (Group I) and cisplatin-treated (Group III) groups, suggesting no adverse effect. These results suggest that GG extract confers nephroprotection in a dose-dependent manner, comparable to the effect of cisplatin. A significant dose-dependent correlation was found for BUN ($r^2=0.7430$; p value=0.0013), creatinine ($r^2=0.04601$; p value=0.0311) and uric acid ($r^2=0.7737$; p value=0.0008) dose at 200mg/kg and 400mg/kg (Figure 3 d).

Electrolyte analysis revealed that *GG* extract exerted a significant protective effect against DLA-induced disturbances in calcium ($Ca^{2+}$) levels ($F_{4,20} = 45.42$, $p < 0.0001$, $\eta^2 = 0.9007$; one-way ANOVA, (Figure 4b) and potassium ($K^+$) ($F_{4,20} = 68.85$, $p < 0.0001$, $\eta^2 = 0.9245$; one-way ANOVA, (Figure 4c). At a dose of 200 mg/kg, the extract significantly restored sodium ($p = 0.0008$) and calcium ($p < 0.0001$) concentrations, while potassium levels ($p < 0.0001$) were notably reduced compared to the tumor-induced group (Group II). Administration of the higher dose (400 mg/kg) further improved the electrolyte profile, with sodium ($p < 0.0001$) and calcium ($p < 0.0001$) levels approaching those of the normal control group (Group I), and potassium levels returning fully to the physiological range. These findings demonstrate a clear dose-dependent effect of *GG* in normalizing electrolyte imbalances associated with lymphoma progression. Regression analysis revealed that GG extract significantly altered electrolyte levels in a dose-dependent manner. Sodium ($r^2=0.4549$; p value=0.0324) and calcium

($r^2$=0.4386; p value=0.0369) showed moderate effects, while potassium showed a stronger association ($r^2$ = 0.6098, p = 0.0077) with increasing dose (Figure 4d).

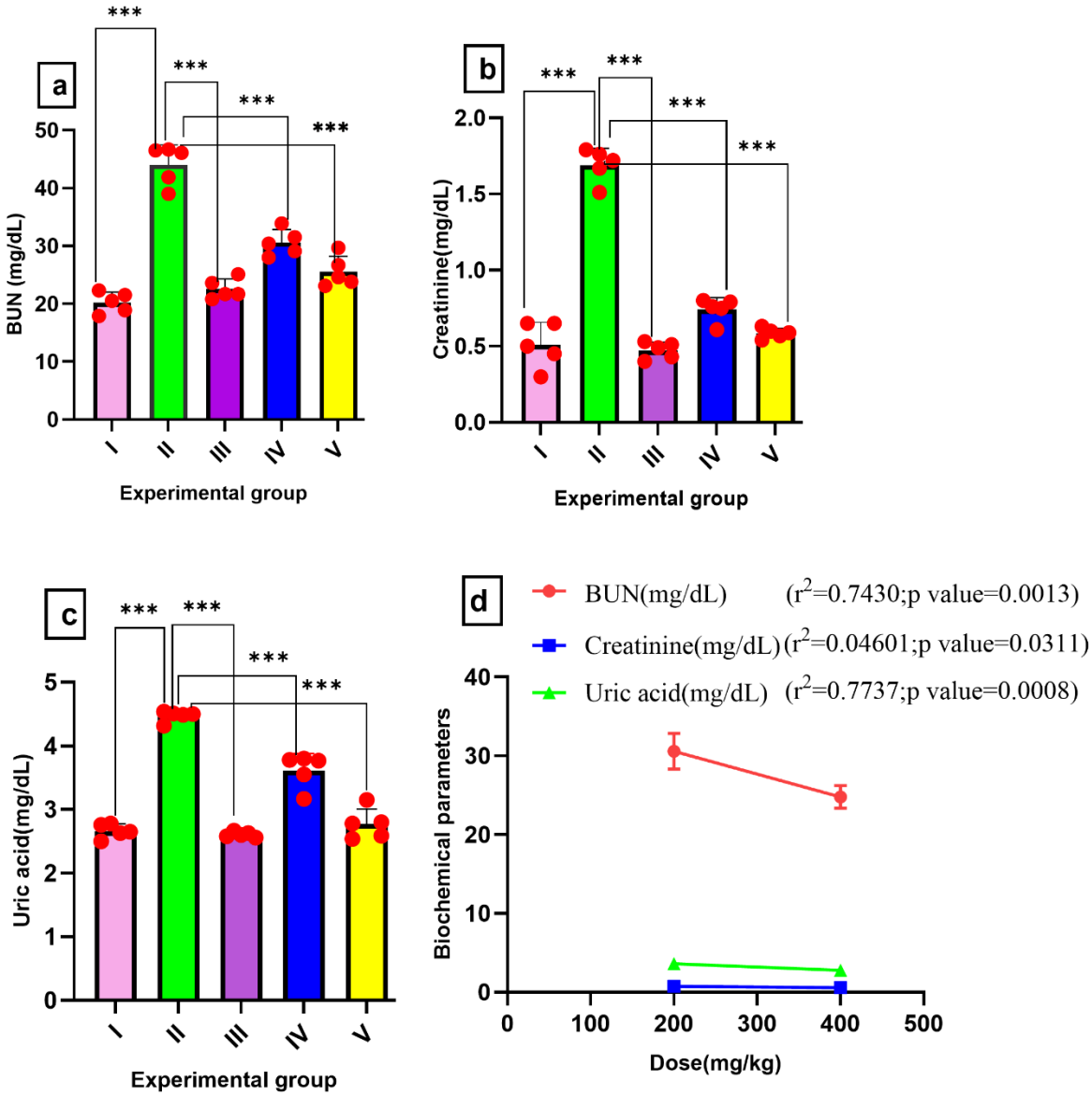


**Figure 3.** Effect of *Gnetum gnemon* extract on serum kidney function markers (BUN, creatinine, and uric acid) in DLA-induced mice in all groups. (a)BUN (b) creatinine (c)Uric acid. (d) Regression analysis showing the dose-dependent effect of leaf extract on serum biochemical parameters in DLA-bearing mice.

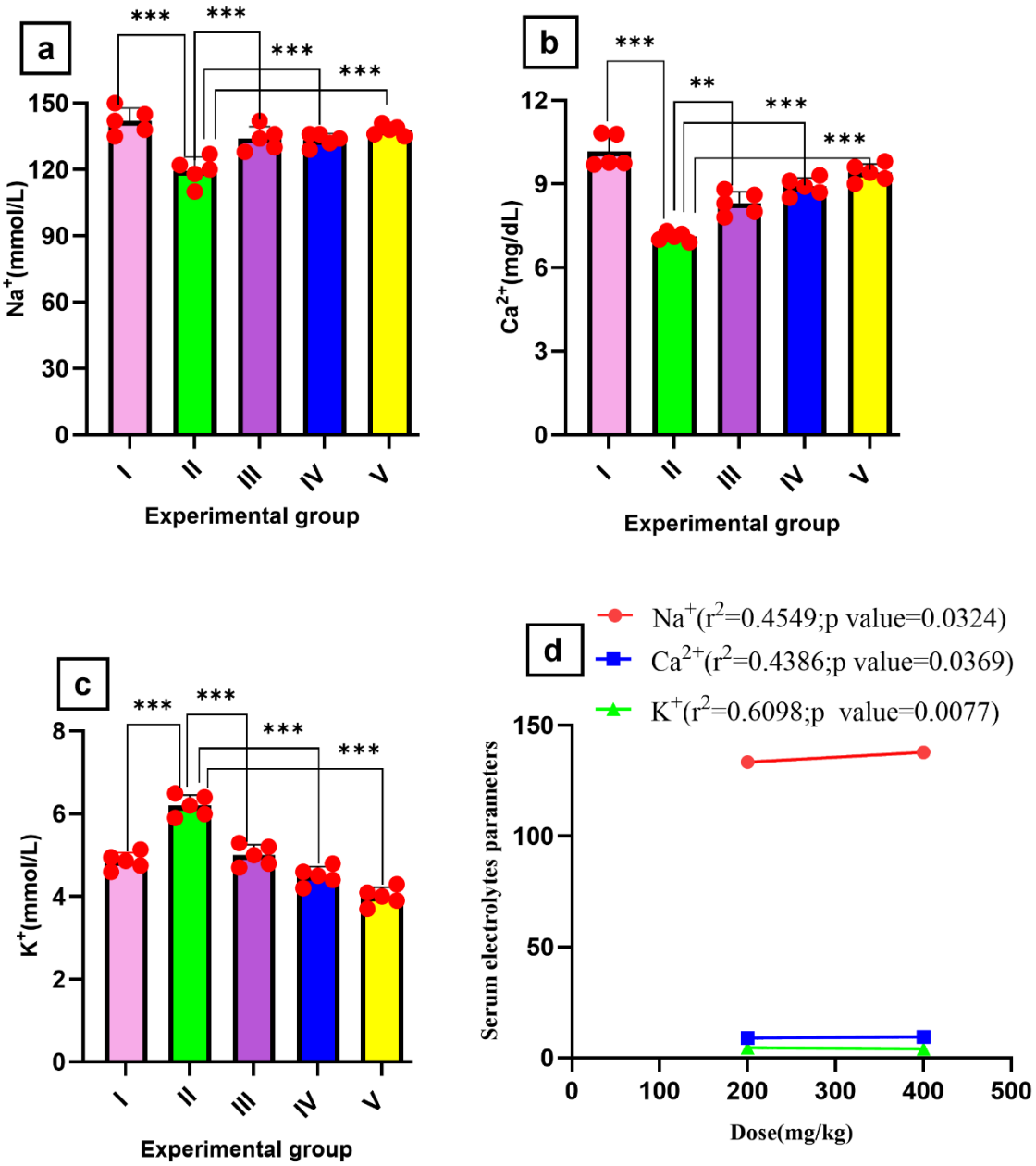


**Figure 4.** Dose-dependent effect of *Gnetum gnemon* extract on serum electrolytes parameters in Dalton's lymphoma-induced mice. This figure illustrates the impact of *Gnetum gnemon* methanolic leaf extract on DLA-induced alterations in serum electrolyte levels-(a)sodium ($Na^+$) (b) calcium ($Ca^{2+}$) and (c)potassium ($K^+$) (d) Regression analysis of serum electrolyte levels ($Na^+$, $K^+$, $Ca^{2+}$) across different doses of leaf extract in DLA-bearing mice.

Changes in oxidative stress markers such as GSH ($F_{4,20}$=66.81, p value <0.0001, $\eta^2$=0.9304 ; one way ANOVA, (Figure 5a), CAT (F4,20=170.5, p value <0.0001, η2=0.9716; one way ANOVA, (Figure 5b) , SOD (F4,20=13.17, p value <0.0001, η2=0.7246 ; one way ANOVA, (Figure 5c ) and MDA ($F_{4,20}$=148, p value <0.0001, $\eta^2$=0.9672 ; one way ANOVA, (Figure 5d) in the experimental groups were significantly, reflecting the level of renal oxidative damage as well as the effects of treatments. All the enzymes of antioxidant defence such as GSH, CAT, SOD, and MDA were found to be within normal range in the control group I, indicating they were still at baseline levels. Group II mice which were induced with Dalton's lymphoma had significant renal toxicity and oxidative stress due to significantly reduced (P<0.001) GSH, CAT, SOD and thereby significantly (P<0.001)

elevating MDA. Cisplatin treatment in Dalton's lymphoma ascites-induced mice (Group III) still had an oxidative stress rate that has a significant improvement ($p<0.001$) in GSH, CAT, and SOD levels, and decrease in MDA as comparing to Group II. Antioxidant biomarkers in mice (Group IV) treated with 200 mg/kg of *GG* extract also showed improvement of all the parameters including GSH, (p value <0.0001) CAT (p value <0.0001), SOD (p =0.0195), MDA ($p<0.0001$) but less than those treated with cisplatin. Treatment with 400 mg/kg *GG* extract (Group V) led to significantly increase GSH ($p<0.0001$), CAT ($p<0.0001$) and SOD ($p<0.0001$) levels and significantly reduced MDA ($p<0.0001$) level. A significant correlation was observed between *Gnetum gnemon* dose and oxidative stress markers, with elevated GSH ($r^2 = 0.7773$; $p = 0.0007$), CAT ($r^2 = 0.8264$; $p = 0.0003$), SOD ($r^2 = 0.5700$; $p = 0.0116$), and reduced MDA ($r^2 = 0.8766$; p value<0.0001), highlighting dose-responsive antioxidant activity (Figure 5e). The findings emphasize that Dalton's lymphoma causes oxidative damage that cisplatin may lessen this damage, and that *Gnetum gnemon* extract offers dose-dependent nephroprotection.

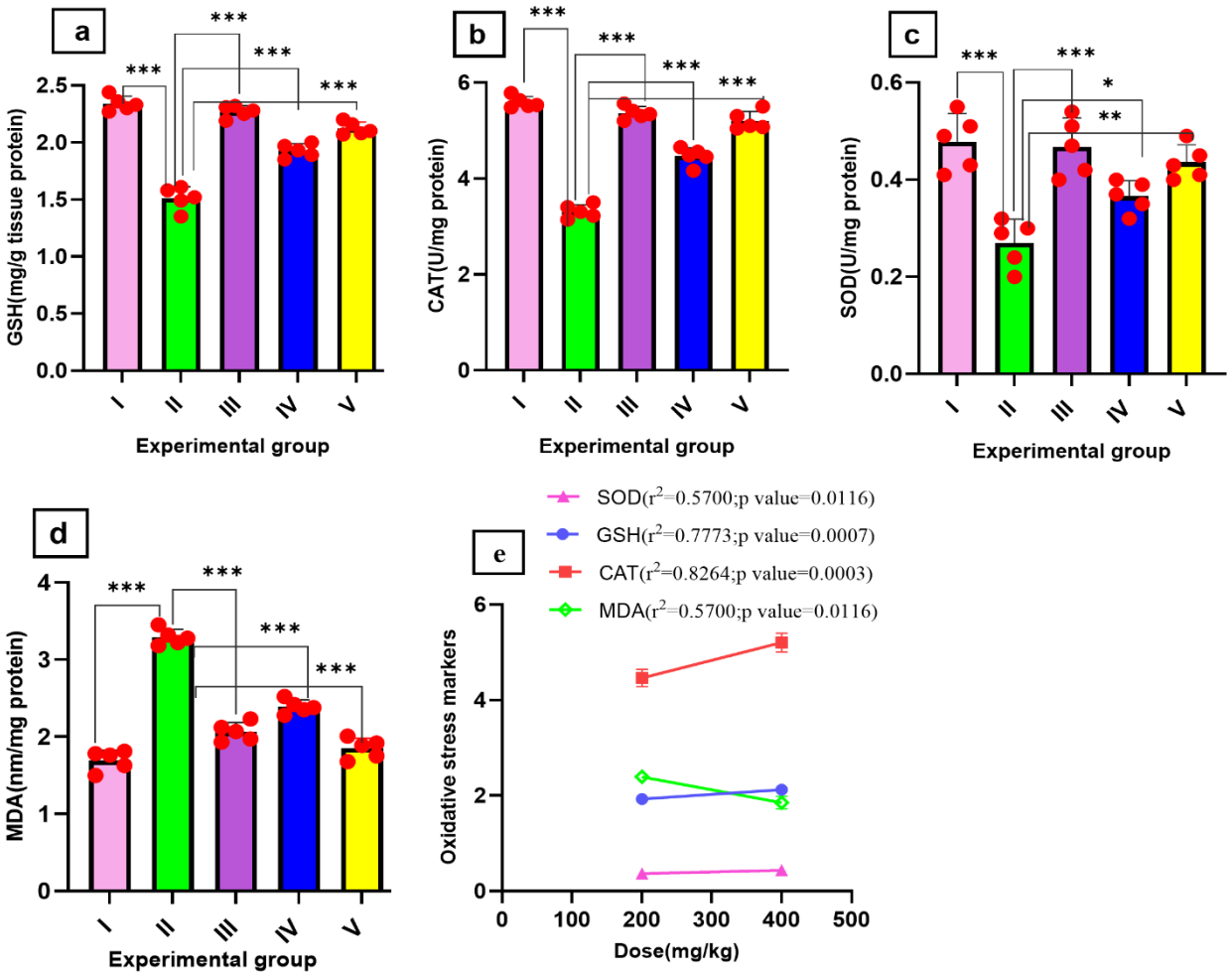


**Figure 5.** Changes in renal oxidative stress markers among experimental groups. (a) GSH (b) CAT (c) SOD and, and (d) MDA levels were measured to assess oxidative stress in DLA-bearing mice and the effects of treatment. (e) Regression analysis of oxidative stress markers in DLA-bearing mice treated with different doses of leaf extract.

Histopathological analysis revealed that the renal architecture of the control group (Group I) remained intact, exhibiting normal glomerular and tubular structures (Figure 6a). In contrast, group II, which received Dalton's lymphoma (DLA), showed signs of lymphoma-induced renal toxicity, including glomerular atrophy, vacuolar degeneration of tubular epithelial cells, tubular necrosis, and intense inflammatory infiltration (Figure 6b). Group III, consisting of DLA-induced mice treated with cisplatin, exhibited reduced inflammation and capsular space, absence of glomerular atrophy, and moderately improved tubular structure (Figure 6c). Mice administered with GG extract at 200 mg/kg body weight (Group IV) showed mild improvement in renal function, as indicated by reduced tubular damage, absence of glomerular atrophy, and minimal inflammation (Figure 6d). Notably, group V, which received GG extract at a higher dose of 400 mg/kg, exhibited near-complete histological recovery of the kidney (Figure 6e). The renal tissue showed well-preserved glomeruli, normal tubular architecture, and minimal to no inflammatory infiltration, suggesting a strong dose-dependent nephroprotective effect of the extract.

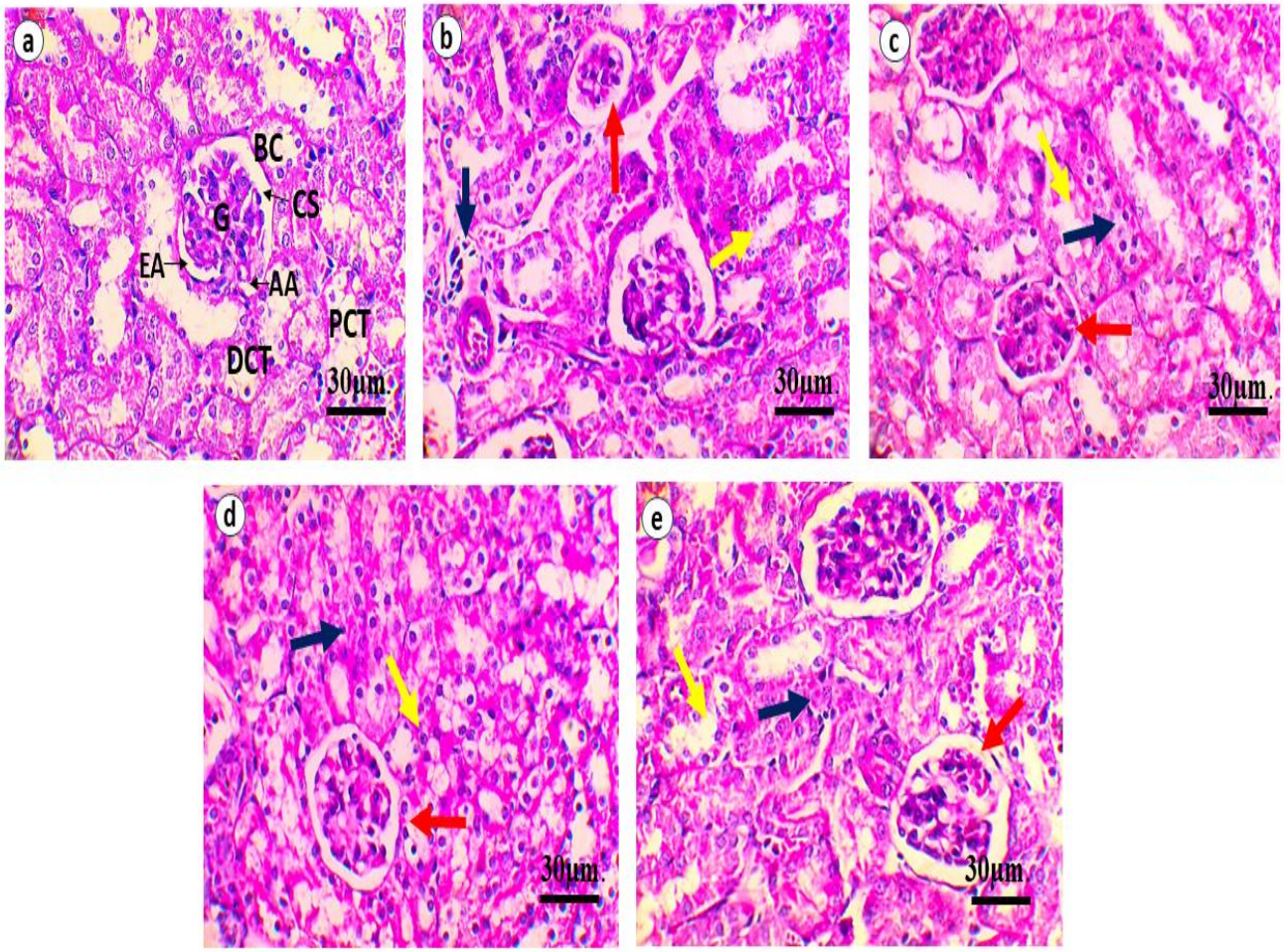


**Figure 6.** Photomicrographs of kidney sections showing their structure under a light microscope: (a) Group I; Healthy kidney with normal glomeruli (G), proximal convoluted tubules (PCT) showing clear brush borders, and distal convoluted tubules (DCT) with open lumens, bowman capsule (BC) afferent arteriole (AA) and efferent arteriole (EA) .(b) Group II ; Significant tissue changes with signs of glomerular atrophy and nepropathy (GA;red arrow), tubular necrosis (TN), and a high number of inflammatory cells (II;black arrow). The brush border in PCT is damaged (yellow arrow). (c) Group III; Some improvement in kidney structure with less tubular damage or necrosis and inflammation (black arrow) and capsular space decreases and no glomerular atrophy (red arrow). (d) Group IV; Partial recovery, showing less damage to tubules, lower inflammation. (e) Group V; Noticeable improvement in kidney structure with minimal necrosis or inflammation (green arrow) and capsular space recovered to normal (blue arrow). Bar-30µm.

Morphometric analysis of the kidneys from normal and DLA-induced mice across all groups revealed significant changes in glomerular number ($F_{4,20} = 38.99$, $p < 0.0001$, $\eta^2 = 0.8863$; one-way ANOVA, (Figure 7a), capsular space area ($F_{4,20} = 51.51$, $p < 0.0001$, $\eta^2 = 0.9115$; (Figure 7b), and glomerular area ($F_{4,20} = 248.2$, $p < 0.0001$, $\eta^2 = 0.9491$;

(Figure 7c). In Group I (normal control), no visible structural alterations in the kidneys were observed. In contrast, the DLA induced group (Group II) exhibited a significant reduction ($p<0.0001$) in glomerular number, attributed to lymphoma-induced nephrotoxicity. Administration of the cisplatin (Group III) significantly increased ($P<0.0001$) the glomerular count compared to the DLA induced group II. *G. gnemon* extract at 200 mg/kg (Group IV) moderately restored glomerular number ($p = 0.0043$), while the 400 mg/kg dose (Group V) produced a more pronounced effect ($p < 0.0001$). Additionally, the glomerular area was significantly ($p<0.0001$) reduced in the DLA induced group II compared to the normal control group I, indicating structural damage from tumor infiltration. Treatment with the cisplatin (Group II, $p<0.0001$) significantly restored glomerular area. Mice treated with *Gnetum gnemon* extract at both 200 mg/kg and 400 mg/kg doses also showed significant improvements in glomerular area ($p < 0.0001$). Capsular space area analysis revealed that DLA-induced nephropathy led to a significant increase ($p<0.0001$) in bowman's capsular space thickness, suggesting glomerular atrophy. Treatment with cisplatin and GG extract at both dosages significantly ($p < 0.0001$) normalized capsular space, indicating preserved nephron integrity and restoration of renal morphology in affected kidneys.

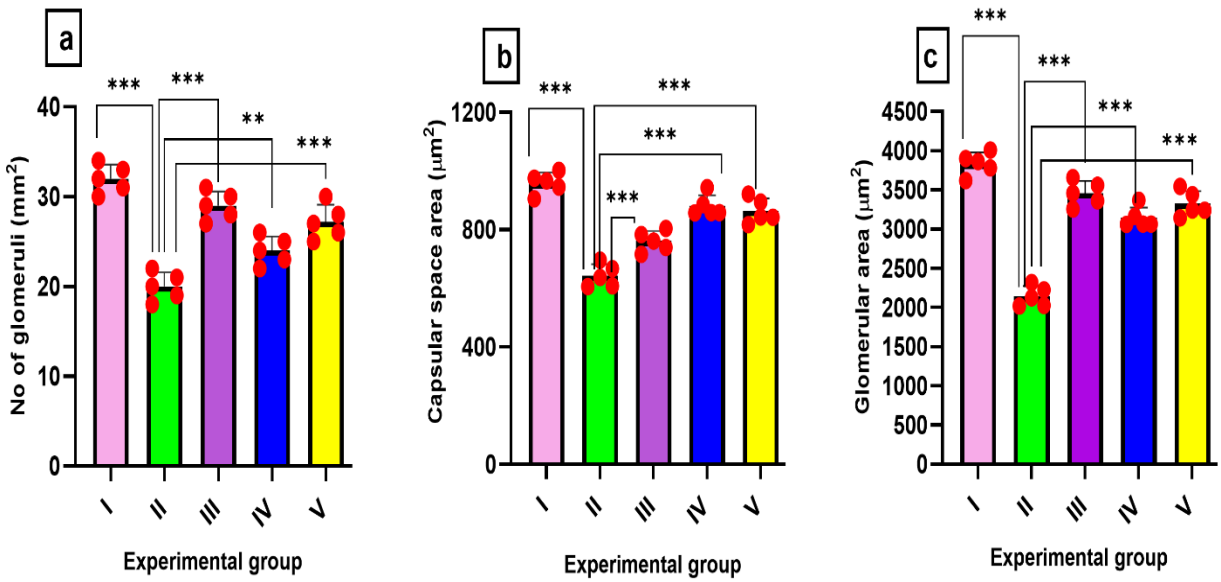


**Figure 7.** Morphometric analysis of kidney tissue parameters in control, DLA-induced, and treatment groups**.**

The present study demonstrates the strong nephroprotective effects of *Gnetum gnemon* methanolic extract (GGME) in mice bearing Dalton's lymphoma ascites (DLA), as evidenced by significant changes across multiple physiological, biochemical, histopathological, and morphometric studies. Acute toxicity testing showed that GGME was safe up to a dose of 2000 mg/kg body weight, with no observed signs of abnormal behavior or mortality during the 14-day observation period. The non-toxic nature of GGME was further confirmed by an estimated $LD_{50}$ greater than 2000 mg/kg body weight, which is consistent with previously reported $LD_{50}$ values exceeding 5000 mg/kg (Yanagihara *et al*., 2012). The absence of acute toxicity supports the use of GGME at 200 and 400 mg/kg for evaluating its nephroprotective potential. Increased body weight in tumor-bearing mice was attributed to rapid tumor progression and accumulation of ascitic fluid in the peritoneal cavity. Compared to normal controls (Group I), DLA-induced mice exhibited a marked increase in body weight. Conversely, mice treated with cisplatin (Group III) showed a significant reduction in body weight. A dose-dependent reduction in weight was also observed in GGME-treated groups (IV and V), suggesting effective tumor suppression. In our experiment, *Gnetum gnemon* extract (400mg/kg) significantly (p value=0.0194) decreased tumor growth, improvement of body weight as well as restored kidney functioning of DLA-bearing mice. The effects indicate that GGME can mitigate the harmful effects of cancer and enhance renal health by mitigating oxidative stress. Similarly, Melinjo seed extract (MSE), a part of *Gnetum gnemon*, also exhibited potent capacity to enhance tumor size, blood vessels development within tumors (angiogenesis), and liver metastasis in colon-26 tumor-bearing mice (Narayanan *et al*., 2015b). Although their study focused on tumor suppression, angiogenesis and metastasis, the improvement in body weight in both models reflects a decrease in tumor burden and cancer-related stress. These two studies corroborate that *G. gnemon* possesses strong antitumor activity. The key difference is that their study was conducted on a colon cancer and investigated metastasis whereas our study was conducted on DLA lymphoma and investigated organ protection and

oxidative stress. This comparison reveals that *G. gnemon* does not only possess direct anticancer activity, it preserves the overall health as the tumor grows. Collectively, these results indicate that *G. gnemon* possesses the potential of being used as an anticancer agent in various forms of cancer.

BUN, creatinine, uric acid levels in the serum are the main indicators used to measure renal function and detect underlying pathological disorders. Under normal physiological condition, the kidney regulate creatinine, urea, and uric acid properly through effective filtration and waste elimination. Creatinine arises from muscle metabolism, while BUN and uric acid produced from purine and protein metabolism respectively, are efficiently filtered and excreted (Ajiboye *et al*., 2024). Biochemical analysis revealed that untreated DLA mice had significantly ($p<0.0001$) elevated levels of BUN, creatinine, and uric acid, potentially linked to tumor-induced oxidative stress. GGME treatment significantly lowered serum levels of BUN and uric acid in tumor-bearing mice while slightly increase level of creatinine, but does not show any toxic effects, indicating improved kidney function. In a related study, treatment with 400 mg/kg of hydroethanolic extracts from *G. africanum* and *G. buchholzianum* resulted in slightly increase in creatinine and urea. However, the kidney tissues showed histologically normal and intact kidney structure, indicating no renal toxicity (Enone *et al*., 2022).

Electrolyte in serum plays a critical role in the operation of the kidney and monitoring pathology is one of its major markers. These electrolytes are carefully balanced in a functioning kidney, so as to maintain physiological equilibrium, which will provide fluid homeostasis, integrity of cellular structure, nerve impulse conductivity, bone health, muscular activity, and blood pH levels(Lewis *et al*., 2015). On the other hand, imbalance of serum electrolytes indicates the presence of an underlying kidney pathology and is frequently noted in disorders, including acute kidney injury (AKI) or chronic kidney disease (CKD). Poor filtration and excretion creating electrolytes can give way to imbalances, e.g., hyperkalemia, which presents a risk of cardiac arrhythmias (Lewis *et al*., 2015). Electrolyte imbalances, commonly associated with nephrotoxicity, were evident in DLA-induced mice. Altered sodium, potassium, and calcium levels indicated renal impairment. Administration of GG methanolic extract, particularly at 400 mg/kg, effectively restored these electrolyte levels to near-normal values.

A substantial decrease in GSH, CAT, and SOD and elevation of MDA, a key indicator of lipid peroxidation were observed in DLA induced -mice presented in Figure 5.The findings support earlier research showing that tumors generate oxidative stress that alters redox homeostasis in kidneys (Su *et al*., 2021). Antioxidants enzymes are crucial for neutralizing reactive oxygen species (ROS) and maintaining the stability of cellular structures (Rao *et al*., 2025). The antioxidant enzymes CAT, GSH, and SOD are essential in neutralizing oxidative damage in kidney tissues, while MDA serves as an indicator of lipid peroxidation(Jena *et al*., 2023).In disease states such as cancer, excessive generation of reactive oxygen species which impairs the antioxidant defense and mechanisms leading to damage in both the structure and function of kidney tissues (Bhatti *et al*., 2022). Notably, administration of GGME effectively reversed oxidative imbalances in a dose dependent manner, offering protection against tumor-induced nephrotoxicity. Similarly(Enone *et al*., 2022)reported that treatment with combination of *Gnetum africanum* and *Gnetum buchholzianum* extracts improved oxidative stress markers by elevating antioxidant enzyme levels (GSH, CAT, and SOD), along with a marked decline in MDA, indicating attenuation of oxidative stress.

Histopathological evaluation confirmed that GGME protected against lymphoma-induced architectural disruption of renal tissues. In untreated DLA induced mice (Group II), the kidneys exhibited severe structural alterations, including glomerular atrophy and tubular degeneration. However, treatment with GGME especially at 400 mg/kg notably improved renal histoarchitecture, reduced inflammatory infiltration, and restored glomerular and tubular integrity to near-normal conditions. These histopathological improvements were consistent with the biochemical results, reinforcing the extract's nephroprotective efficacy. Such protective effects of plant-based treatments on histopathological alterations have been well documented, with numerous studies demonstrating their ability to enhance antioxidant defenses, mitigate oxidative stress, and protect against tissue damage caused by toxins or diseases (Musik, 2020; Allagui *et al*., 2023). Morphometric analysis of kidney tissues further supported the protective role of GGME. Treatment with the extract, particularly at the higher dose, resulted in increased glomerular count, restoration of glomerular area, and normalization of capsular space area. These positive outcomes are consistent with earlier reports indicating that plant-derived antioxidants promote tissue repair and shield organs from structural damage in response to oxidative and inflammatory stress (Chansiw *et al*., 2019). Although previous research suggests that GG leaf extract possesses kidney-protective properties in degenerative disorders, this is the first study to evaluate its nephroprotective effects in DLA-induced nephrotoxicity. Our findings provide a strong basis for future investigations into its potential as a therapeutic agent.

## CONCLUSION

This study highlights the potential of *Gnetum gnemon* methanolic extract as a nephroprotective agent against Dalton's lymphoma-induced nephrotoxicity in Swiss albino mice. The extract demonstrated strong protective effects across a range of physiological, biochemical, and histological parameters, with a dose-dependent response. Further studies are warranted to elucidate the precise mechanisms of action and to assess its clinical applicability. The use of *Gnetum gnemon* as a complementary therapeutic agent in managing cancer-induced nephrotoxicity holds promise and merits deeper exploration.

**ACKNOWLEDGMENT**

The authors express sincere thanks to the head of the Department of Zoology, Cotton University, Guwahati for the facilities provided to carry out this research work.

**CONFLICT OF INTERESTS**

The authors declare no conflict of interest

**ETHICS APPROVAL**

All animal experiments conducted in this study received approval from the Institutional Animal Ethics Committee of Cotton University, Guwahati (Regn. No. 15/IAEC/CU/05/01/2021) and were performed in compliance with the guidelines of the CPCSEA.

**FUNDING**

This study received no specific funding from public, commercial, or not-for-profit funding agencies.

**AI TOOL DECLARATION**

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

**DATA AVAILABILITY**

Data will be available on request

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