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Research Article

Effect of Ethanolic Fraction of *Anogeissus Leiocarpa* on Renal Function in Wistar Rat

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Abstract

Objective : This study investigated the effects of acute administration of ethanolic extract of *Anogeissus leiocarpa* on renal function in Wistar rats.

Materials and Methods : Wistar albino spawn and *Anogeissus leiocarpa* root bark were used as biological material for this study. The method employed was to administer single doses of ethanolic extract of *Anogeissus leiocarpa* root bark orally. Urine and blood samples were collected followed by measurement of renal markers (blood and urine urea and creatinine levels, urea and creatinine clearance).

Results: Serum urea and creatinine levels were unchanged compared with controls. In contrast, extract and furosemide caused a significant ($p < 0.001$), dose-dependent increase in urinary creatinine. Ethanolic extract of *Anogeissus leiocarpa*, at doses of 300 and 500 mg/kg bw, significantly increased creatinine and urea clearance and thus glomerular filtration rate.

Conclusion : Levels and clearances of urea and creatinine, kidney biomarkers, were not altered. This study shows that the extract is not nephrotoxic.

Keywords : *Anogeissus leiocarpa*, glomerular filtration rate, nephrotoxic, urea, creatinine

INTRODUCTION

The use of plants in primary health care is not new. Indeed, since ancient times, medicinal plants and plant-based recipes have been a major source for the treatment of many human and animal diseases throughout the world. With good reason, more than 80% of the local population in developing countries still rely on plants or plant-based recipes for primary health care, according to the World Health Organization¹. This enormous upsurge in interest, although mostly due to their accessibility, cost and the ancestral beliefs of populations, is above all reinforced by the idea that what is natural can only be beneficial. However, given the complexity and diversity of the biomolecules they contain, medicinal plants can have undesirable or even toxic effects². The risk is all the greater for noble organs such as the liver, heart and kidney, in the absence of precautions for use³.

For the latter organ, they can lead to renal failure or a worsening of pre-existing renal failure⁴. Indeed, in the event of prolonged prescription of plants well known for their

therapeutic virtues such as *Juniperus communis*, *Taraxacum officinale*, *Petroselinum crispum*, *Asparagus officinalis*, *Medicago sativa*, they can lead to acute tubular necrosis and electrolyte disorders⁵. Although the proportion of renal pathologies attributable to medicinal plants is not well documented, in Côte d'Ivoire hepatonephritis accounted for 16% of plant poisoning cases admitted to intensive care⁶. Consequently, the issue of nephrotoxicity due to medicinal plants deserves particular attention.

With this in mind, the aim of the present study was to evaluate in vivo the effects of acute administration of the ethanolic fraction of *Anogeissus leiocarpa* on glomerular filtration rate (GFR), one of the parameters used to assess overall renal function.

MATERIALS AND METHOD

I-1- Materials

I-1-1 Biological material

I-1-1-1 Plant material

Anogeissus leiocarpa root bark was used. These organs were harvested in January 2013 in Kouto (Bagoué region), a town located 725 km north of Abidjan (Côte d'Ivoire). Authentication of the plant species harvested was carried out by the late Professor AKE ASSI Laurent, using the herbarium of the Centre National Floristique (CNF) at the Université Félix Houphouët Boigny.

I-1-1-2 Animal

White albino rats of the species *Rattus norvegicus* of the Wistar strain, of both sexes, two to three months old and weighing between 170 and 200 g were used. The animals came from the animal house of the Life and Earth Sciences Laboratory of the Ecole Normale Supérieure d'Abidjan (ENS). For their acclimatization, they were kept for two weeks in plastic cages with stainless steel covers containing wood shavings bedding renewed every two days. The animals were regularly fed standard rat pellets, and received tap water in stainless steel bottles as drinking water.

I-2- Method

I-2-1 Experimental design

Thirty (30) nulliparous, non-pregnant male and female rats aged two to three months and weighing between 170 and 200 g on average were used. These rats were deprived of food and water for 18 hours. Prior to experimentation, the rats' bladders were emptied by gentle compression of the pelvic region⁷.

Each of these rats received orally 2.5 mL/100 g bw of isotonic saline (NaCl 0.9%) to impose a uniform water load. Forty-five (45) minutes later, these rats were randomly divided into five batches of 6 rats each. Orally, the control lot received 1 mL of distilled water, while the furosemide lot received 1 mL of furosemide at dose of 20 mg/kg bw. Animals in the experimental batches (ETHA 100, ETHA 300 and ETHA 500) each received 1 mL ETHA at doses of 100, 300 and 500 mg/kg bw respectively.

I-2-2 Glomerular filtration rate

After 24 h, the rats' blood is collected by caudal amputation. Urine samples were taken from jars previously placed under each cage. Urine and plasma creatinine and urea levels were then determined using the CYANStart automated system according to the manufacturer's instruction manual, with appropriate reagents. Creatinine clearance, a reflection of glomerular filtration, was determined according to the formula proposed by **Dizaye et al.**⁸:

$$\text{Creatinine Clearance } (\mu\text{L}/\text{min}) = \frac{U \times V}{P \times T}$$

U: urinary creatinine level

P: Plasma creatinine level

T: urine collection time (time equal to 24 h)

V: volume of urine collected at 24 h

I-2-3 Statistical analysis

Analysis and graphical representation of the data were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). The values expressed are the means of at least three experiments, together with the standard error of the mean (Mean \pm SEM). The significance of the difference was assessed by one-way analysis of variance (ANOVA) followed by Dunnett's non-parametric multiple comparison test. The difference was considered statistically significant if the p-value < 0.05.

RESULTS

II-1- Effect of ETHA on plasma urea levels after one day of treatment

The effect of ETHA on plasma urea levels is shown in figure 1. Compared with plasma levels in control animals (0.76 ± 0.12 g/L), plasma urea levels in animals treated with furosemide at 20 mg/kg bw (0.82 ± 0.04 g/L) and those treated with ETHA at 100 (0.85 ± 0.08 g/L), 300 (0.70 ± 0.04 g/L) and 500 mg/kg bw (0.73 ± 0.08 g/L) did not vary significantly ($p > 0.05$).

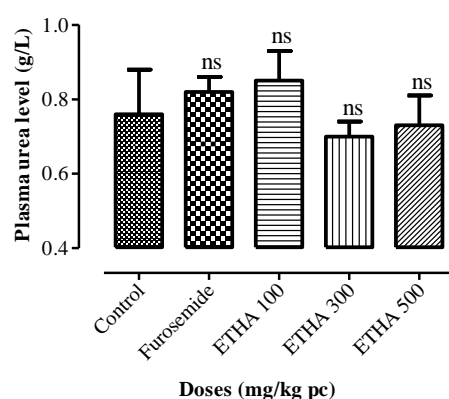


Figure 1: Effect of the ethanolic fraction of *Anogeissus leiocarpa* on plasma urea and creatinine levels after 24 h treatment.

Values are averages affected by the standard error of the mean ($m \pm esm$). Each batch comprises 6 animals ($n=6/\text{batch}$). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

II-2- Effect of ETHA on plasma creatinine after one day of treatment

Figure 2 shows the effect of ETHA on plasma creatinine levels. Administration of ETHA doses ranging from 100 to 500 mg/kg

bw to rats did not alter creatinine levels. Furosemide, in contrast to ETHA, significantly ($p < 0.001$) increased creatinine levels from 14.03 ± 0.37 mg/L (control) to 18.35 ± 0.22 mg/L, an increase of 30.79% compared with the control batch.

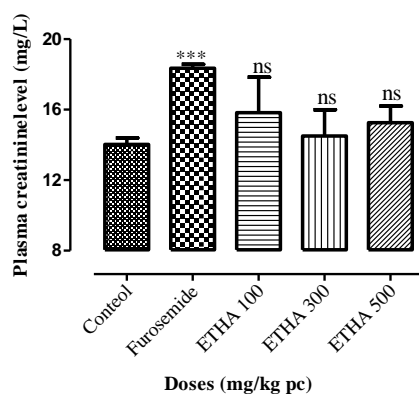


Figure 2: Effect of the ethanolic fraction of *Anogeissus leiocarpa* on plasma creatinine levels after 24 h of treatment.

Values are averages affected by the standard error of the mean ($m \pm esm$). Each batch comprises 6 animals ($n=6/\text{batch}$). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

III-3- Effect of ETHA on urinary urea levels after one day's treatment

The effect of ETHA on urinary urea levels is shown in figure 23. This figure shows that the urinary urea level of animals in the control batch is 0.39 ± 0.00 g/L. This compares with 0.54 ± 0.09 g/L for the ETHA-treated batch at 100 mg/kg bw and 0.52 ± 0.03 g/L for the ETHA-treated batch at 300 mg/kg bw,

representing a significant increase ($p < 0.001$) in urinary urea levels of 38.46% and 33.33% respectively compared with rats in the control batch. The urinary urea levels of animals in batches treated with ETHA at 500 mg/kg bw (0.46 ± 0.05 g/L) and those treated with furosemide at 20 mg/kg bw (0.44 ± 0.05 g/L) did not vary significantly ($p > 0.05$) from those of rats in the control batch (0.39 ± 0.00 g/L).

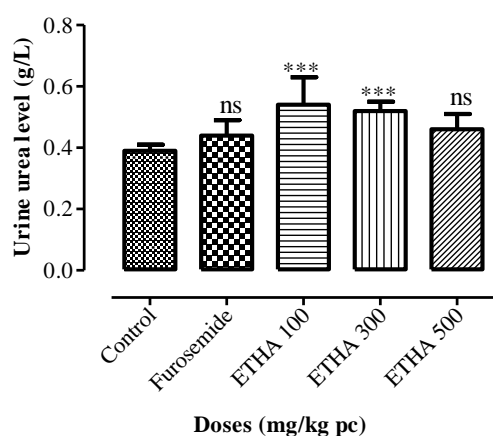


Figure 3: Effect of ethanolic extract of *Anogeissus leiocarpa* on urinary urea concentration after 24 h treatment.

Values are averages affected by the standard error of the mean ($m \pm esm$). Each batch comprises 6 animals ($n=6/\text{batch}$). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

III-4- Effect of ETHA on urinary creatinine after one day of treatment

Figure 4 shows the variation in urinary creatinine levels in animals treated with ETHA at doses ranging from 100 to 500 mg/kg bw and furosemide at 20 mg/kg bw. The figure shows that these different substances produced a significant ($p < 0.001$) and dose-dependent increase in urinary creatinine

levels. The urinary creatinine level in control animals was 750.13 ± 19.38 mg/L. This rose to 809.80 ± 117.90 at 100 mg/kg bw, 984.60 ± 95.99 at 300 mg/kg bw and 1583.03 ± 247.80 mg/L at 500 mg/kg bw, corresponding to respective increases of 7.95, 31.25 and 111%. In furosemide-treated animals, urinary creatinine was 1303.02 mg/L, an increase of 73.73%.

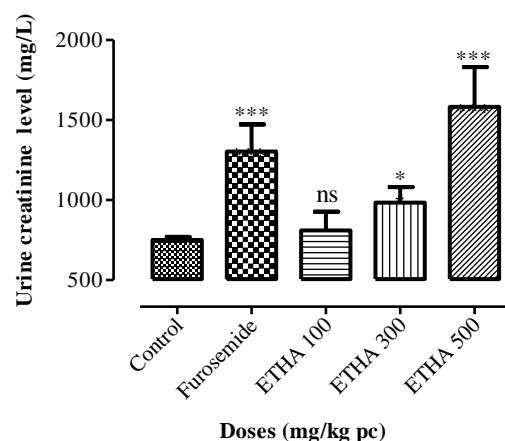


Figure 4: Effect of the ethanolic fraction of *Anogeissus leiocarpa* on urinary creatinine levels

Values are averages affected by the standard error of the mean ($m \pm esm$). Each batch comprises 6 animals ($n=6$ /batch). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

II-5- Effect of ETHA on creatinine clearance

Figure 5 shows the evolution of creatinine clearance in animals from the control batch and those from batches treated with ETHA and furosemide. The figure shows that creatinine clearance in control animals was $63.11 \pm 12.36 \times 10^{-3} \mu\text{L}/\text{min}$. In animals from batches treated with ETHA and furosemide, creatinine clearance increased significantly ($p < 0.05$) and in a dose-dependent manner. In animals treated with furosemide at

a dose of 20 mg/kg bw, creatinine clearance was $207.11 \pm 38.77 \times 10^{-3} \mu\text{L}/\text{min}$, an increase of 228.17% compared with the control. ETHA administration increased creatinine clearance from $63.11 \pm 12.36 \times 10^{-3} \mu\text{L}/\text{min}$ (control) to $73.22 \pm 11.49 \times 10^{-3} \mu\text{L}/\text{min}$ (100 mg/kg bw), $227.91 \pm 26.03 \times 10^{-3} \mu\text{L}/\text{min}$ (300 mg/kg bw) and $396.57 \pm 62.30 \times 10^{-3} \mu\text{L}/\text{min}$ (500 mg/kg bw). The percentage increases were 16.02, 261.13 and 528.38% compared with the control lot, for animals treated with ETHA at 100, 300 and 500 mg/kg bw respectively.

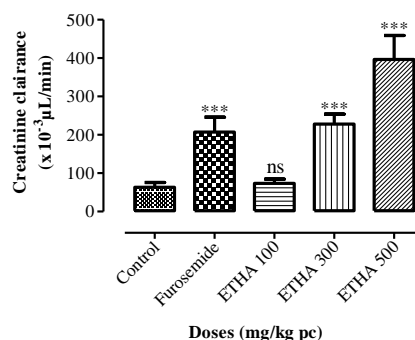


Figure 5: Creatinine clearance in rats treated with the ethanolic fraction of *Anogeissus leiocarpa* and furosemide

Values are averages affected by the standard error of the mean ($m \pm esm$). Each batch comprises 6 animals ($n=6$ /batch). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

II-6- Effect of ETHA on urea clearance

Figure 6 shows the evolution of urea clearance in animals from the control batch and those from batches treated with ETHA and furosemide. The figure shows that urea clearance in control animals was $0.61 \pm 0.1 \times 10^{-3} \mu\text{L}/\text{min}$. Urea clearance in animals treated with furosemide at 20 mg/kg bw was $0.95 \pm 0.08 \times 10^{-3} \mu\text{L}/\text{min}$, an increase of 140%. It was significantly higher than in control animals ($p < 0.05$). Like furosemide, ETHA at doses of

300 and 500 mg/kg bw significantly increased urea clearance ($p < 0.001$). Urea clearance in animals treated with ETHA at 300 mg/kg bw ($1.34 \pm 0.38 \times 10^{-3} \mu\text{L}/\text{min}$) and 500 mg/kg bw ($2.05 \pm 0.21 \times 10^{-3} \mu\text{L}/\text{min}$), corresponded to percentage increases of 119.67 and 236.06% respectively. The clearance of animals treated with ETHA at 100 mg/kg bw ($0.68 \pm 0.2 \times 10^{-3} \mu\text{L}/\text{min}$) was not significantly different from that of control animals ($p > 0.05$) ($0.61 \pm 0.1 \times 10^{-3} \mu\text{L}/\text{min}$).

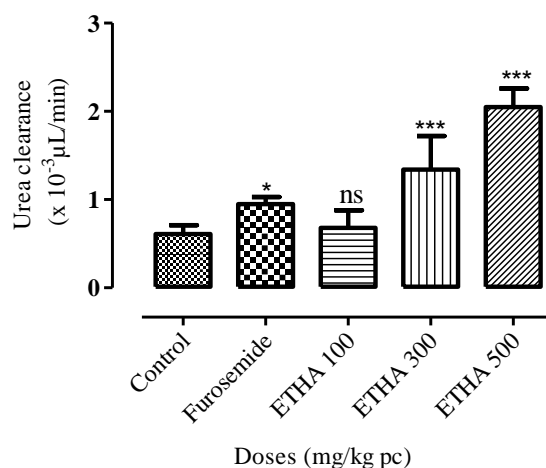


Figure 6: Urea clearance in rats treated with the ethanolic fraction of *Anogeissus leiocarpa* and furosemide

Values are averages affected by the standard error of the mean ($m \pm \text{esm}$). Each batch comprises 6 animals ($n=6/\text{batch}$). ns: there is no significant difference from the plasma urea level of the control batch at $p > 0.05$. ETHA: ethanolic fraction of *A. leiocarpa*, Furosemide: batch treated with furosemide at 20 mg/kg bw. ETHA 100: batch treated with ETHA at 100 mg/kg bw, ETHA 300: batch treated with ETHA at 300 mg/kg bw. ETHA 500: batch treated with ETHA at 500 mg/kg bw.

DISCUSSION

Like all the body's noble organs, the kidneys contribute to homeostasis through a number of vital functions, including acid-base balance, regulation of electrolyte balance in the blood, elimination of metabolic waste products, secretion of certain enzymes and hormones, metabolism and regulation of blood pressure⁹. Thus, any functional or structural abnormality could have an impact on certain renal parameters such as urea, creatinine and electrolytes...Blood urea and serum creatinine, the traditional markers of nephrotoxicity and renal dysfunction¹⁰, were used to report on kidney status in this study.

At the doses employed, serum urea and creatinine levels were unchanged from controls after one day of treatment (Figure 1 and 2). In contrast to ETHA, furosemide significantly ($p < 0.001$) increased creatinine levels by 30.79% compared to control. Serum creatinine, a measurable by-product of muscle metabolism, is an excellent indicator of renal health. These results probably suggest the absence of any functional damage to the kidney, showing that the fraction does not interfere with the kidney's ability to excrete metabolites. Kolawole et al.¹¹ also raised this hypothesis with the aqueous extract of *Hibiscus sabdariffa*. Indeed, nephrotoxicity is indicated by a significant rise in serum creatinine and urea levels¹⁰. According to Imo et al.¹², creatinine retention in the blood is evidence of renal failure. Furthermore, Imo et al.¹³ indicate that any increase in serum urea levels could be due to acute glomerulonephritis although several other causes can be cited such as nephrosclerosis and tubular necrosis. For several authors, renal diseases that decrease the glomerular filtration rate of urea lead to its retention in the blood¹⁴.

Urine urea and creatinine levels were also assessed. The results showed that the different substances (ethanolic extract and furosemide) produced a significant ($p < 0.001$) and dose-dependent increase in urinary creatinine levels (Figure 4). This suggests that these substances fully eliminate excess creatinine and urea. The results in Figure 3 show that at doses of 100 and 300 mg/kg bw, the urinary urea level increased, whereas at 500 mg/kg bw, there was no significant change, as with furosemide at 20 mg/kg bw. This study therefore shows that at low doses of the extract, urea, the end product of protein and amino acid

metabolism, is eliminated to a much greater extent. This observation was also made by Imo et al.¹³ when evaluating the effects of ethanolic extracts of leaves, seeds and fruits of *Datura metel* L. on the renal function of male albino rats. According to some authors, glomerular filtration of urea or creatinine from the blood into the urine is the main mechanism involved in eliminating excess nitrogen from the body. These results suggest that the chemical constituents of the extract studied do not cause damage to renal structure and function. These phytomolecules would therefore preserve the integrity of the kidneys by eliminating waste products from the main metabolisms, while avoiding their accumulation in the blood.

In the present study, glomerular filtration was assessed through urea and creatinine clearance, as was done by Pablo-Pérez et al.¹⁵ in their work with *Eysenhardtia polystachya* extract. The clearance of a substance (creatinine, urea) measures the ratio between the rate of elimination of the substance by the kidneys (through urine) and its concentration in the blood.

Indeed, creatinine is the indirect marker of choice for the assessment of glomerular filtration rate (GFR)¹⁶. Results obtained in this study, showed that after 24 h, ethanolic fraction of *A. leiocarpa*, at doses of 300 and 500 mg/kg bw caused a significant increase in creatinine (Figure 5) and urea clearance (Figure 6). These results suggest that the fraction increases glomerular filtration in rats treated with these doses. These metabolic wastes would therefore be eliminated from the body following the increase in their excretion rate at high doses of the extract. These results are similar to those of Maghrani et al.¹⁷ with the aqueous extract of *Retama raetam* and those of Néné-Bi et al.¹⁸ obtained with the aqueous extracts of *Bridelia ferruginea*. These results confirm the good elimination of urea and creatinine through urine.

CONCLUSION

The ethanolic fraction of *Anogeissus leiocarpa* at the doses used did not alter the biochemical parameters (serum and urine urea and creatinine levels, urea and creatinine clearance) used to assess renal function in this study. The results showed that the fraction studied had no effect on renal structure and function. Furthermore, they showed that metabolic waste products are eliminated from the blood through the urine by increasing the

rate of glomerular filtration. The use of *Anogeissus leiocarpa*, like many other plants in the rich medicinal plant heritage, would therefore be safe for the kidneys.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Islam SU, Dar TU, Khuroo AA, Bhat BA, Mangral ZA, Tariq L, Tantray WW, Malik AH. DNA barcoding aids in identification of adulterants of *Trillium govanianum* Wall. ex D. Don. J. Appl. Res. Med. Aromat. Plants, 2021 ; 23(10):03-05. <https://doi.org/10.1016/j.jarmap.2021.100305>
- Khasmi M.EL, Farh M. Impact des plantes médicinales sur le rein. RMN, 2022 ; 2(5):32-40
- N'zoue KS, Kouame NM, Yobo BMS, Kamagate M, Die-Kakou H, N'Guessan D. Effets néphrotoxiques des plantes médicinales en Côte d'Ivoire. MAN, 2020 ; 67(4):193-201
- Nidal AJ, Abdel NZ, Rowa AR, Malik AA, Fatim H, Zakaria H et al. Ethnopharmacological survey of medicinal plants practiced by traditional healers and herbalists for treatment of some urological diseases in the West Bank/Palestine. BMC Complement Altern Med, 2017 ; 17:255. DOI: <https://doi.org/10.1186/s12906-017-1758-4>
- Naouaoui S. Néphrotoxicité des plantes médicinales. Thèse de médecine, Faculté de médecine et de pharmacie. Université de Marrakech, Maroc, 2019 ; 162p.
- Die-Kacou H, Kamagate M, Yavo JC, Daubrey T, Diafouka F. Intoxication par les plantes à Abidjan: Difficultés du diagnostic étiologique et aspects ethnobotaniques. Bio Africa; 2009 ; 7:34-43
- Jayakody JRAC, Ratnasooriya WD, Fernando WANA, Weerasekara KR. Diuretic activity of leaves extract of hot water infusion of *Ruta graveolens* L. in rats. J Pharmacol Toxicol, 2011; 1-8. DOI: <https://doi.org/10.3923/jpt.2011.525.532>
- Dizaye KF, Alberzingi BO, Sulaiman SR. Renal and vascular studies of aqueous extract of *Urtica dioica* in rats and rabbits. Iraqi J Vet Scis, 2013 ; 27(1):25-31. DOI : <https://doi.org/10.33899/IJVS.2013.82948>
- Stevens LA, Coresh J, Greene T, Levey AS. Assessing kidney function- Measured and estimated glomerular filtration rate. N Engl J Med. 2006;354:2473-83. DOI: <https://doi.org/10.1056/NEJMra054415>
- Al-Naimi MS, Rasheed HA, Hussien NR, Al-Kuraishy HM, Al-Gareeb AI. Nephrotoxicity: Role and significance of renal biomarkers in the early detection of acute renal injury. J Adv Pharm Technol Res. 2019 ; 10(3):95-99. doi: https://doi.org/10.4103/japtr.JAPTR_336_18 . PMID: 31334089; PMCID: PMC6621352.
- Kolawole OT, Akiibinu MO, Akanji MA. Assessment of the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on some biochemical indices of renal function in rats. Int J Pharma Sci, 2014;4(3):587-590.
- Imo C, Uhegbu F. Renal Protective Effect of Ethanolic Leaf Extract of *Gongronema latifolium* Benth in Acetaminophen-induced Renal Toxicity in Male Albino Rats. Am Chem Sci J, 2015; 8(3):1-10. DOI: <https://doi.org/10.9734/ACSJ/2015/18881>
- Imo C, Arowora KA, Ezeonu CS, Yakubu OE, Nwokuwu CD, Azubuike NC, Sallah YG. Effects of ethanolic extracts of leaf, seed and fruit of *Datura metel* L. on kidney function of male albino rats. J Tradit Complement Med. 2017;28:9(4):271-277. doi: <https://doi.org/10.1016/j.jtcme.2017.09.001> .
- David MS, Jeff SC, Nigel B, David WJ, Glenda CG. Oxidative stress, antioxidant therapies and chronic kidney disease. Nephrology, 2012; 17:311-321. DOI: <https://doi.org/10.1111/j.1440-1797.2012.01572.x> .
- Pablo-Pérez SS, Estévez-Carmona MM, Meléndez-Camargo ME. Diuretic activity of the bark of *Eysenhardtia polystachya*. Bangladesh J Pharmacol, 2016; 11:212-217. DOI: <https://doi.org/10.3329/bjp.v11i1.24659>
- Hougardy JM, Delanaye P, Le-Moine A, Nortier J. L'estimation de la filtration glomérulaire en 2014: intérêts et limites des tests et formules. Rev Med Brux, 2014; 35:250-256.
- Maghrani M, Zeggwagh NA, Haloui M, Eddouks M. Acute diuretic effect of aqueous extract of *Retama raetam* in normal rats. J Ethnopharmacol, 2005; 99(1):31-35. DOI: <https://doi.org/10.1016/j.jep.2005.01.045>
- Néné-Bi SA, Soro TY, Zahoui OS, Traoré F. Effet d'une administration aiguë d'un extrait aqueux de *Bridelia ferruginea* Benth. (Euphorbiaceae) sur la fonction rénale chez le rat. Phytothérapie, 2013; 11:359-364. <https://doi.org/10.1007/s10298-013-0817-3>