WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Phytochemical Constituents and Renal Biochemical Effects of Methanol Leaf Extract of *Datura stramonium* in Levonogestrel-Induced Nephrotoxicity in Albino Rats

Amen Ananias[1], Dawoye Yusufu[2], Israel Ahmadu Kiledu[3] and Gaddafi Suleiman[4]

[1234]Department of Biochemistry and Chemistry, Federal Polytechnic Bali

*amenananias@mail.com

ABSTRACT :

Kidney toxicity is becoming a global problem. The used of medicinal plants in the treatment of nephrotoxicity is gaining wider attention. The aim of this research was to evaluate the phytochemical constituent and renal biomarkers effects of methanol leaf extract of Datura stramonium in Levonogestrel-induced nephrotoxicity in Albino rats. The leaves of Datura stramonium were extracted by cool maceration using methanol as solvent and was assessed for its nephroprotective properties at different concentration (100 mg/kg b.w, 200mg/kg/b.w and 300mg/kg/b.w). Twenty five (25) rats made up five groups (n=5). 0.75mg of the drug was administered across 4 groups. Phytochemical screening of the extract showed the presence of Alkaloids, reducing sugars, Phenolic, Tannins, and Anthraquinones, quinones, Fixed oil and Saponines, while Flavonoids was negative. Na^+ concentration was significantly ($P< 0.05$) restored by plant extract (147.93 ± 2.04^a and 148.16 ± 1.50^{ab}) following the drug-induced toxicity (112.04 ± 74.71^{ab}). The concentration of Urea, Creatinine, K^+ and Cl^- were not significant ($P< 0.05$) among the groups. However, there was moderate elevation following extract administration after kidney intoxication. The plant extract therefore has demonstrated a nephroprotective properties by ameliorating the effect of kidney intoxication.

Keywords: *Datura stramonium*, Kidney, phytochemical screening, Drug, Nephrotoxicity

Introduction

Kidney plays an important part in the maintenance of our endocrine and acid-base balance, blood pressure, erythropoiesis and many others. Kidney toxicity is one of the most common kidney problems and occurs when body is exposed to a drugs, chemicals or toxins (Esmail and Talab, 2019). Irrespective of the level of exposure, there is need to detect the presence of harmful chemicals and toxins in living system, and the concept of intoxication is related to specific organ alterations and clinical symptoms (Fernando and Antonio, 2001). One of the methods used to measure the level of exposure to xenobiotics (drugs, harmful chemicals), and its potential impacts on living organisms, including human beings is by monitoring with biological markers. According to Puntma (2009), "Biomarkers (biological markers) are characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention". Any biological indicator that can be tested can be used as a biological marker. These biomarkers can be cellular or molecular and body fluids like the Blood, Urine, Saliva, serum, cerebrospinal fluid etc., are used to determine them (Srinitha, 2021). Most of the commonly used clinical tests on biomarkers are biochemical, which provides soluble biomarkers while physiological test provides anatomical and functional biomarkers (Puntman, 2009). Biomarkers are increasingly being used as a primary warning in a perceived pathological condition. They are produced in organs, tissues and cells that are diseased, in response to various pathological states or as an outcome of an individual's reaction to drug taken. Biomarkers play an important role in clinical diagnosis because its early identification reduces damage to the body (Bodaghi *et al*., 2023). Biomarkers provide valuable information on the pathological condition of an individual. It is grouped based on their main clinical application, such as diagnostic, monitoring, pharmacodynamics/ response, predictive, prognostic, safety, and susceptibility/ risk biomarkers (Michel, 2022). Pharmacodynamic biomarkers, for instance, are markers of a specific pharmacological response and are of good interest in dose optimization studies (Michel, 2022). Blood biomarkers are useful in early diagnosis of Alzheimer's disease. Blood biomarkers offer high sensitivity and specificity for Alzheimer's disease. Blood biomarkers are a potential solution as they are much easier and cheaper to deliver (Coulthard & Hosseini, 2023). Biomarkers are the basis for all in-vitro diagnosis (Srinitha, 2021). There are cardiovascular biomarkers, bone and joint Biomarkers, hormonal biomarkers, renal biomarkers, (Cañadas-Garre *et al.,* 2021). Biomarkers provide useful information to healthcare experts for diagnosing and managing inflammatory conditions, monitoring treatment effectiveness, and predicting disease outcomes. Inflammatory biomarkers measure and assess the seriousness, presence, progression of inflammatory processes within the body (Varahalarao & Sandeep, 2024). Another important biomarker is the nutritional biomarker which could provide important information on the association between diet and health. Biomarkers of nutritional exposure and biomarkers of nutritional status are very useful in validating dietary measurement (Dolores & José, 2015). Other forms of nutritional biomarkers are the Recovery biomarkers (based on the concept of metabolic balance between intake and excretion over a given period), Concentration biomarkers (based on the metabolism of individual characteristics like age, sex etc.), Replacement biomarkers (referring specifically on compound whose nutritional facts

are not adequate) and predictive biomarkers (based on dose-response relationships with intakes) (Dolores & José, 2015). There are also genomic biomarkers which measures DNA or RNA that could be an indicator of normal biologic processes, pathogenic processes, and response to therapeutic or other interventions (Ankeet *et al*., 2020). Tumor biomarkers exist which is of great importance in clinical cancer management (Zhou *et al*., 2024).
Bulk of drugs administered orally go into the gastrointestinal tract (GIT), get absorb into the bloodstream through the alveoli to the liver where it gets metabolize before reaching the target site. Abused of drugs inflict damage on the kidney where most drug metabolites are excreted (Graham, 2013). Medicinal plants elucidate their pharmaceutical properties on biomarkers by reducing or lowering the effect of an inducing compound or pathological conditions. Some demonstrate immunomodulatory benefits as such could possess potential lead compound for the development and design of immunomodulatory drugs (Joshua *et al*., 2022).
Datura stramonium is a plant with an herbaceous base that is branched and glabrous, reaching up to an approximate level of 1 m in height (Sharma *et al*., 2021). It is known as thorn apple, Jamestown or devil's trumpet in English and Nchuagwo in Igbo (joy *et al*., 2021). Traditionally, *Datura stramonium* is reported to have been used in treating different inflammatory diseases (Bakht *et al*., 2022). The plant possesses Neurological effects, Anti-obesity, Antioxidative effects, Anti-infection, Anti-viral activity, Rhizogenic activity, Anticholinergic activity, Immune modulatory effect, Tropinone-reductase activities, Enzyme activities, Lectin properties, Bronchodilator effects etc. (Sepide, 2016). All the plant parts are used for medicinal purposes, though seeds and leaves are mostly used (Egyptian Drug Authority, 2022). The plant is also credited with insecticidal, repellent, and organophosphate protective activity, Larvicidal, Antifungal activities, Acaricidal, oviposition deterrent properties and anticancer activity (Ali, 2017, Kaur *et al*., 2020). Reports of Phytochemicals of the leaf extract showed the presence of alkaloids, phenols and saponins (Ikponmwosa *et al*., 2022). Levonogestrel is known by other names as postcoital contraceptive or morning after pill (Kahlenborn *et al*., 2015). The drug is indiscriminately consumpt due to unprotective sex by young female adults. The issue concerning its long time usage at metabolizing and major excretory site is crucial.

Justification of the Study

Drug-induced l kidney injury is the leading cause of death in many countries in sub Saharan African countries. The multiple and frequent use of Levonorgestrel as contraceptive is on the increase by females both unmarried and the married. This could affect the excretory organ, the kidneys. Therefore, it is imperative to investigate the effects of this drug the kidney using animal models like the albino rats.

Significance of Study

The indiscriminate use of any pharmaceutical agent constitutes an abuse and leads to a negative pharmaceutical effect ("site effect") at the target site and the entire system. The consumption of levonorgestrel is rampant, being carried away by the immediate remedy it offers, neglecting its effects on the kidney. The drug leaflets from the manufacturers do not carry useful information in many cases, on the effects of its long-time usage on the kidney. Also, there are just a few related literatures and research work done in that regard. The aim of this research is to investigate the Phytochemical Constituents and Renal Biochemical Effects of Methanol Leaf Extract of *Datura stramonium* in Levonogestrel-Induced Nephrotoxicity in Albino Rats.

Methodology

Chemicals and reagents

Chemicals and reagents of Standard-grade were purchased from reputable vendors for use in the experiment.

Biological materials

Fresh leaves of *Datura stramonium* and albino rats (Wistar) are the biological materials employed in this research. Mature leaf of *Datura stramonium* was harvested at Anguwan NEPA and Anguwan Tiv in October 2024 within Bali metropolis, and certification was done in plant science department of Modibbo Adama University, Yola.

Animal care

Wistar albino rats numbering 25 (mixed sex) within standard weight were procured from the Department of Animal Science, Modibbo Adama University Yola. The rats were kept for two weeks to get acclimatized to the new environment in the Animal House, located in the biological garden, behind the SLT department of Federal Polytechnic Bali. The animals were kept at 25^0C with unlimited access to clean water and commercial animal feed was provided.

Sample preparation

Following certification, leaves were washed under running water and dried under shed for 30 days before grinding into smooth powder and storing in an air-tight bottle. Cool maceration method by Khadayat *et al.* (2020) was adopted in extract preparation. One hundred and fifty gram (150g) of powdered leaf was soaked in 850mls of methanol placed in a tight container with constant agitation for 72hours. Followed by evaporation of the solvent in a water bath.

Phytochemical analysis

Qualitative phytochemical analysis was carried out on the methanol leaf extract of datuar stramonium using standard procedures to identify the phytochemical constituents according to methods reported by Juniad and patil (2020).

a) **Alkaloids** :(Wagner's test). Two (2) drops of Wagner's reagent was added to 5 mL filtrate of the leaf extract along the sides of test tube. A brown precipitate was detected.

b) **Carbohydrates** :(Seliwanoff's Test). One milliliter (1mL) of the leaf extract solution was added to 3mL of seliwanoff's reagent and heated on water bath for 1 min. A rose red colour was observed

c) **Reducing sugars**: (Benedict's test). Zero point five milliliter (0.5mL) of the leaf extract filtrate was added to the same volume of Benedict's reagent and boiled for 2min. Green colour was observed.

d) **Phenolics**: Two Grams (2grms) of the leaf extract was dissolved in 5mL distilled water and 3mL of 10% lead acetate solution was added. A white precipitate was formed.

e) **Tannins** :(Gelatin test). One gram (1grm) of the leaf extract was dissolved in 5mL of distilled water, 1% gelatin solution was added with 10% NaCl. A white precipitate showed the presence of tannins.

f) **Flavonoids: (**Alkaline reagent test). One milliliter (1mL) of the methanol leaf extract was added to 2mLs of 2% NaOH solution with 3 drops of dilute HCl. An intense yellow colour, becomes colourless on addition of diluted acid.

g) **Anthraquinones :(** Ammonium hydroxide test). Five milligrams (5mg) of the leaf extract was dissolved in isopropyl alcohol and 2 drops of conc. ammonium hydroxide solution was added. Formation of red colour was observed after 2 min.

h) **Quinones:** (concentrated sulphoric acid test). Ten milligram (5mg) of the leaf extract was dissolved in 2mls of isopropyl alcohol, 2 drops of con.H_2SO_4 was added. A red colour indicates the presence of Quinone.

i) **Fixed oil**: (Stain test). Little quantity of the leaf extract was pressed in between two filter papers. Oil stain was observed on the paper.

j) **Saponins:** (Foam test). Zero point 2 grams (0.2gm) of the leaf extract was added to 5mL distilled water; shaken well and heated to boiling. The appearance of creamy miss of small bubbles was observed.

Experimental design

After two weeks of adaptation, the 25 albino rats were grouped into 5 groups of 5 rats each.

- Group 1: Normal control (given feed and water only)
- Group 2: 0.75mg of levonorgestrel (inducer only)
- Group 3: 0.75mg levonorgestrel+ 100 mg/kg b.w extract
- Group 4: 0.75mg levonorgestrel+ 200 mg/kg b.w extract
- Group 5: 0.75mg levonorgestrel+ 300 mg/kg b.w extract

All administration was done orally.

Sample collection from experimental animals

After 21 days of extract administration, rats were starved all night and Whole blood was collected into plan sample bottles by cardio puncture according to IACUC (2022) guidelines for further analysis.

Renal Function Indices

Serum concentration of Urea and Creatinine, along with Cl-, Na+ and K+ were determined using standard methods.

- **Determination of Serum Urea Concentration:** This was assessed using the method described by Fawcett and Scout (1960).
- **Determination of Serum Creatinine Concentration:** The colorimetric method was used to determined serum creatinine concentration according to Tietze *et al*. (2006).
- **Determination of Serum Cl^- Concentration:** This was done according to the method of Tietze *et al*. (2006).
- **Determination of Serum Na^+ Concentration:** This was done according to the method of Tietze *et al*. (2006).
- **Determination of Serum K^+ Concentration:** This was done according to the method of Tietze *et al*. (2006).

Statistical Analysis

All data obtained were expressed as mean± SD. One way ANOVA was used to test for difference among groups using SPSS version 27. Post Hoc test was used to test for significant differences 1among group mean. A p- value of < 0.05 was considered statistically significant.

Results and discussion

Results

Table 1: Selected phytochemicals in Datura stramonium methanol leaf extract.

phytochemical	Present	Absent
Alkaloids	+	
Carbohydrates	++	
Reducing sugars	+	

Phenolic	++	
Tannins	++	
Anthraquinones	+	
Flavonoids		-
Quinones	+	
Fixed oils	+++	
Saponins	+	

+++ (Highly abundant), ++ (Abundant), + (Minute) and – (Absent)

The result of kidney biomarkers of levonogestrel intoxication revealed significant increase ($p<0.05$) in the Na^+ concentrations in the extract administered groups (147.93 ± 2.04^{a}) and (148.16 ± 1.50^{ab}) as compared to the drug control (112.04 ± 74.71^{ab}) as shown in table 2. However, the concentration Na^+ decreased significantly ($p< 0.05$) as a result of treatment with the extract in each of the groups when compared with the normal control. While the concentration of Urea, Creatinine, K^+ as well as Cl^- were not significant ($p<0.05$) among the groups, however, relative changes were ameliorated by the plant leaf extract.

Table 2: Effects of Methanol Leaf Extract of *Datura stramonium* on Some Kidney Biomarkers of Levonorgestrel-induced Albino Rats.

Group	Normal Control	Drug Control	Drug+100mg/kg extract	Drug+200mg/kg extract	Drug+300mg/kg extract
Urea (mg/dl)	2.00 ± 00.01^{a}	1.5 ± 1.00^{a}	1.99 ± 0.01^{a}	0.99 ± 1.12^{a}	1.99 ± 0.03^{a}
Creatinine (mg/dl)	40.80 ± 0.42^{a}	30.69 ± 20.45^{a}	40.00 ± 0.20^{a}	20.12 ± 23.23^{a}	40.55 ± 0.31^{a}
Cl^- (mg/dl)	91.75 ± 3.12^{a}	69.46 ± 46.30^{a}	93.99 ± 1.12^{a}	46.34 ± 53.51^{a}	92.45 ± 2.37^{a}
Na^+ (mg/dl)	254.91 ± 216.56^{a}	112.04 ± 74.71^{ab}	147.93 ± 2.04^{a}	72.93 ± 84.22^{ab}	148.16 ± 1.50^{ab}
K^+ (mg/dl)	4.28 ± 0.07^{a}	3.49 ± 2.34^{a}	5.22 ± 0.18^{a}	2.66 ± 3.07^{a}	5.06 ± 0.27^{a}

Results are expressed as mean ± SD (n=5). Significant at $p< 0.05$ compared with control using analysis of Variance.
Groups with same superscript in the row are not significantly different
Group with different superscripts in the same row are significantly different.

Discussion

Datura stramonium is a medicinal plant, known for its health benefits on broad range of diseases. The medicinal properties of this plant is attributed to its numerous important phytochemicals present in different parts of the plant. These metabolites are present in different parts of the plant like the leaf, stem, roots, flowers, stem bark, etc. the presence of these phytochemicals play important role in the survival of the plant itself by fighting against competitors, diseases and predators. Others includes growth control, regulation of pollination, and fertilization among many (Fatemeh *et al*., 2022).some literatures have reported the presence of carbohydrates, oils and major phytochemicals like Alkaloids, Tannins, phenolic compounds, and Flavonoids are reported to be found in the leaf extract of plant under study (Sharma *et al*., 2021; Joy *et al*., 2021;Ikponmwosa *et al*.,2022).

Kidney is one of the vital organ of the human body, because it controls water balance, maintains electrolytes balance, eliminates nitrogenous waste and regulates blood pressure in the body. Kidney is prone to injuries and damage either through mutation or from chronic diabetes, inflammatory diseases, abnormal accumulation of mineral elements, leading to distortion of its functions. These malfunctions are evidently observed in the accumulation of urea and other nitrogenous substances in the blood. The drug administered lowered the serum concentration of Creatinine, urea and electrolytes (Na^+, K^+, and Cl^-). Drugs and other chemicals can be toxic to kidney from the products of incomplete metabolism of drugs and other foreign compounds circulating in the blood. The prevalence of kidney dysfunction has increase recently despite the advance technologies applied in the treatment. However, a lot of experimental studies have indicated that herbal medicine have important effect on improving kidney function (Fatemeh *et al*., 2022).

From the result on table 1 above, it is generally observed that the drug poses a risk to the functionality of the kidney as creatinine and urea serum concentration decreases along with Na^+, K^+, and Cl^- concentration. The level of Urea and Creatinine in serum are clinically used as indicators for determination of kidney function. However, serum creatinine concentration is a more effective indicator than urea concentration at the early stages of kidney disorder. The result also revealed the nephron-restorative properties of methanol leaf extract of *Datura stramonium*. Rui *et al*, (2022), reports the deleterious effects of drugs on the kidney. Which results in the interference of the pathways aiding smooth nephrogenic function. Apart from Levonogestrel with potential renal dysfunction potential, gentamicin, paracetamol, profenofos, D-galactosamine, chronic-stress, sepsis, and cytotoxic drugs can induced kidney injury as well as streptozotocin induced diabetic nephropathy, and chemically induced nephrolithiasis (Esmail and Talab, 2019). Plant secondary metabolites exhibits important biological activities on the kidney. Normal electrolytes, creatinine and urea concentration was restored following the administration of the plant methanol leaf extract due to the nephron- protective properties of metabolites like alkaloids, Tannins etc. Rui *et al*, (2022) reports the nephrogenic protection of alkaloids on electrolytes and kidney biomarkers. Terpenoids and phenolic have also been found to possess neprogenic protective properties (Fatemah *et al*., 2022). The renal protective properties of *Datura stramonium* leaf extract against drug-kidney toxicity also agrees with Alum *et al*, (2023), who reports on the nephroprotective effects of *Datura stramonium* leaf extract against methotrexate nephrotoxicity.

Conclusion

The leaf extract of *Datura stramonium* showed a significant effect in ameliorating the effects of levonogestrel intoxication on the kidney cells of albino rats. Therefore, the leaf extract may be useful in alleviating kidney abnormality as observed from the result.

Conflicts of interest

No conflict of interest declared by the authors

Funding

This is an institutional based research of federal polytechnic Bali, funded by Tetfund.

REFERENCES

1. **A**li E. A. (2017). Medical importance of Datura fastuosa (syn: Datura metel) and Datura stramonium - A review. *IOSR Journal of Pharmacy.* 7 (2); 43-58.
2. Alum, E.U, Famurewa, A.C, Orji, O.U, Aja P.M, Nwite, F, Ohuche, S.E, Ukasoanya, S.C, Nnaji, L.O, Joshua, D., Igwe, K.U, Chima, S.F.(2023). Nephroprotective effects of *Datura stramonium* leaves against methotrexate nephrotoxicity via attenuation of oxidative stress-mediated inflammation and apoptosis in rats. Avicenna Journal of Phytomedicine. 13(4): 377-387.
3. Ankeet S, Dominic C. G, & Brant A. I (2020). Classification of Molecular Biomarkers. SIUJ .1(1): 1-8.
4. Bakht, N., Ashraf, U. K, Muhammad, W.B, Yusuf, S. A, Muhammad, F and Ihsan-U. H, (2022). Datura stramonium Leaf Extract Exhibits Anti-inflammatory Activity in CCL4-Induced Hepatic Injury Model by Modulating Oxidative Stress Markers and iNOS/Nrf2 Expression. *Hindawi BioMed Research International*. 2022:1-20.
5. Bodaghi A., Nadia .F. & Ali R. (2023). Biomarkers: Promising and valuable tools towards diagnosis, prognosis and treatment of Covid-19 and other diseases. *Heliyon*. 9:1-18.
6. Cañadas-Garre, M., Smyth, L., Neville, C., Vijayakumar, A., Kee, F., Woodside, J., & McKnight, A. J. (2021). Biochemical biomarkers. In F. Kee, C. Neville, B. McGuinness, & R. Hogg (Eds.)
7. Coulthard E., & Akram A.H. (2023). Blood biomarkers: ready for clinical practice? *Journal Neurol Neurosurg Psychiatry*.0:1–2. Doi: 10.1136/jnnp-2022-330958.
8. Dolores C, & José M. O (2015): Biomarkers: background, classification, and guidelines for applications in nutritional epidemiology. *Nutricion Hospitalaria*. 31(3):177-188.
9. Egyptian Drug Authority (2022). Pharmacopoeial wild medicinal plants. Egyptian Herbal Monograph. Datura stramonium.164-177.
10. Esmail, A. A. & Talab, T. A. (2019). A review of medicinal plants with nephroprotective effects. *GSC Biological and Pharmaceutical Sciences,* 08(01), 114–122.
11. Fatemeh, R., Maryam, S.M., Rahele, D., Roya, K., & Elahe, E. (2022). Phytochemical Classification of Medicinal Plants Used in the Treatment of Kidney Disease Based on Traditional Persian Medicine. *Evidence-Based Complementary and Alternative Medicine*. https://doi.org/10.1155/2022/8022599.
12. Fawcett, J.K and Scott, J.E. (1960). A rapid and precise methods for determination of urea. *Journal of clinical pathology.* 13(2), 156-157.
13. Fernando G. & Antonio P. (2001). Biomarkers as Biological Indicators to Xenobiotic Exposure. Department of Legal Medicine and Toxicology. University of Granada.
14. Graham L. P. (2013). An Introduction to Medicinal Chemistry. Fifth edition. Oxford University Press.
15. Ikponmwosa O B., Ugbenyen A., & Imarhiagbe O. (2022). Phytochemical Analysis of Datura stramonium (Solanaceae) Leaves using Aqueous and Ethanolic Extracts. *JOJ Wildlife & Biodiversity.* 2022: 4(5): 1-5.
16. Institute of Animal Care and Used Program (2022). Blood collection: the rat.
17. Joshua P. E., Yahaya J., Daniel E. E., Joyce O.O., Arome S.O., Rita O.A., Samson A. O, Olasupo S. A. (2022). Modulation of Immunological Responses by Aqueous Extract of *D a t u r a stramonium* L. Seeds on Cyclophosphamide-Induced Immunosuppression in Wistar Rats. DOI: https://doi.org/10.21203/rs.3.rs-738146/v1
18. Joy, N. D., Chiletam, N. A. & Chizaram, W. N., (2021). Antimicrobial and phytochemical evaluation of Datura Stramonium (Jimsonweed) on selected microorganisms. International Journal of Science and Research Archive, 2021, 02(02), 245–256
19. Junaid, R. S., & Patil, M.K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020; 8(2): 603-608.
20. Kahlenborn C., Rebecca P., & Walter B. S. (2015). Mechanism of action of levonorgestrel emergency contraception. *The Linacre Quarterly* 82 (1); 1-17.
21. Kaur S., Neeraj P., & Shallu (2020). Phytochemistry and Pharmacological Properties of Datura stramonium: An Analysis. *Indian Journal of Pure and Applied Bioscience*. 8(3); 92-105.

22. Khadayat, K, Bishnu P. M, Hira G, Sajani G & Niranjan P., (2020). Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clinical Phytoscience*. 6:34-42.

23. Michel H. (2022). Classification and Applications of Biomarkers. Biomarkers Journal. 8(3); 124.

24. Puntmann V.O. (2009). How-to guide on biomarkers: biomarker definitions, validation, and applications with examples from cardiovascular disease. *Postgraduate Medical Journal*.85:538–545.

25. Rui, Y., Sheng, L., Fei, L., Dan, L., Rong, L. & Nan, Z. (2022). Several Alkaloids in Chinese Herbal Medicine Exert Protection in Acute Kidney Injury: Focus on Mechanism and Target Analysis. Oxidative Medicine and Cellular Longevity. https://doi.org/10.1155/2022/2427802.

26. Sepide M. (2016). Datura stramonium: An updated review. Der Pharma Chemica.8 (17):253-257.

27. Sharma M, Inderpreet D, Kusum R, Anil K.D & Prashant K, (2021). Phytochemistry, Pharmacology, and Toxicology of Datura Species—A Review. *Antioxidants*. 10:1291-1303.

28. Srinitha B. (2021). Biomarkers and disease Diagnosis. Primary Health Care. 11(4): 375-376.

29. Tietze, N.W, Carl, A.B, Edward, R.A & David, E.B., (2006). Tietze Textbook of clinical Chemistry and Molecular Diagnostics. 4th Edition, Elsevier Saunders.

30. Varahalarao V, & Sandeep K, (2024). A Systematic review of Inflammatory biomarkers, Clinical significance, Detection, and their therapeutic agents. *World Journal of Biology Pharmacy and Health Sciences*. 17(03); 242–251.

31. Zhou Y., Lei T., Jiahao Q., Jing X., Xinyu Y., Yu Z., Xinyu T., Xinqi G., Xiaobo C., & Yinglan Z. (2024). Tumor biomarkers for diagnosis, prognosis, and targeted therapy. *Signal Transduction and Targeted Therapy* .9:132-216.