NEPHROPROTECTIVE AND DIURETIC ACTIVITY OF *C. INDICUM* LEAVES EXTRACT ON EXPERIMENTAL ANIMALS

Seema Gupta [1], Neelam Pawar [2], Pravin Bapurao Jadhav [3], Trinayan Deka [4], Sunil Kumar Yadav[5], Koushik Narayan Sarma [6], Shaik. Aleesha [7], Anila Mishra [8]*

1. *Professor, R.R College of Pharmacy Dasarahalli Main Rd, Raja Reddy Layout, Medaralli, Chikkabanavara, Hesaraghatta, Karnataka, Pin Code: 560090*
2. *Assistant Professor, Chaudhary Bansi Lal University, Bhiwani, Haryana, Pin Code: 127021*
3. *Assistant Professor, K. B. H. S. S. Trust Institute of Pharmacy Malegaon District Nashik, Maharashtra, Pin Code: 423105*
4. *Assistant Professor, Faculty of Pharmaceutical Science, Assam Down Town University, Panikhati, Guwahati, Assam, Pin Code: 781026*
5. *Assistant Professor, SRM Institute of Science and Technology, Delhi-NCR Campus, Modinagar Ghaziabad, Uttar Pradesh, Pin Code: 201204*
6. *Assistant professor, Faculty of Pharmaceutical Science, Assam Down Town University, Panikhaiti, Guwahati, Assam, Pin Code: 781026*
7. *Assistant Professor, Dattakala College of Pharmacy, Swami Chincholi, Bhigwan, Pune, Maharashtra, Pin Code: 413130*
8. *Assistant Professor, Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow Campus, Near Malhaur Rly Station, P.O. Chinhat, Lucknow, Uttar Pradesh, Pin Code: 226028*

Corresponding Author: Anila Mishra
Designation and Affiliation: Assistant Professor, Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow Campus, Near Malhaur Rly Station, P.O. Chinhat, Lucknow, Uttar Pradesh, Pin Code: 226028
Email Id: anila.mishra899@gmail.com

KEYWORDS

Kidney, Diuretic, C. indicum, Gentamycin, Furosemide

ABSTRACT

Investigating the nephroprotective and diuretic effects of C. indicum ethanol extract at two doses of 250 and 500 mg/kg bw on gentamycin-induced toxicity in rats was the goal of this investigation. Gentamicin (100 mg/kg) was administered to all animal groups to assess nephroprotective effect; acute renal dysfunction is demonstrated by markedly elevated serum creatinine, urea, and uric acid, as well as lower body weight and numerous histological abnormalities. At doses of 250 and 500 mg/kg, treatment with C. indicum has demonstrated a significant ($p<0.05$, $p<0.01$ and $p<0.001$) dose-dependent improvement in body weight. It has also demonstrated a significant improvement in kidney protection against oxidative stress. Additionally, it was found that, in comparison to the toxic group, treatment with C. indicum considerably reduced the levels of serum creatinine, uric acid, and urea. Serum creatinine, total protein, kidney weights, and body weights were among the parameters that showed nephroprotective effect of EECI treatment when compared to the standard group (Vitamin E, 250 mg/kg) and control group animals against the toxic control group. The protective effect of EECI was further demonstrated by the histological investigations. Additionally, two doses of the C. indicum ethanol extract had a substantial diuretic effect by raising levels of both total urine production and urinary electrolytes including potassium and sodium. Ultimately, these findings indicate that C. indicum ethanol extract has nephroprotective properties against gentamycin-induced nephrotoxicity and strong diuretics effect in rats.

INTRODUCTION

The most prevalent kidney issue is nephrotoxicity, which happens when the body is exposed to a toxin or medicine [1]. Reduced urine concentrating capacity, tubular proteinuria, lysosomal enzymuria, and mid-glucosuria, as well as decreased ammonium excretion, a decrease in glomerular filtration rate, creatinine clearance, an increase in serum blood urea nitrogen, and a change in the morphology of kidney tissue, are functional manifestations of nephrotoxicity. Numerous medications, chemicals, and heavy metals can have a negative impact on the kidney, causing nephritic syndrome, chronic intestinal nephritis, and acute renal failure, all of which can end in irreversible renal damage [2]. However, poor renal function also has a direct impact on cardiovascular health. An aminoglyciside antibiotic called gentamicin is used to treat serious infections. Gentamicin's nephrotoxicity has historically been seen as a tubulopathy, where renal insufficiency is primarily caused by tubular destruction and malfunction. Proteinuria, enzymuria, and electrolytic changes are a few clinical observations that could be explained by this [3, 4].

Drugs called diuretics make the kidneys release more water and salt from the body. Their main impact is to decrease the filtrate's reabsorption of sodium and chloride, which increases water loss from salt excretion. Either directly affecting nephron cells or indirectly altering the filtrate's composition can do this. Technically speaking, "diuresis" denotes a rise in urine volume, while "natriuresis" denotes an increase in renal salt excretion. [5] Because they usually also increase water excretion, important natriuretic medicines are usually referred to as diuretics; the rise in sodium excretion is assumed.[6].

Since the beginning of time, medicinal plants have been utilised extensively as diuretics and to treat a variety of renal disorders. [7, 8] Animal models have demonstrated the diuretic properties of a number of ethnomedical herbs. [9] However, the effectiveness and safety of these plants for their purported medical use have not been fully examined and will need more research. The preparation methods used by traditional healers are typically not standardised and typically do not adhere to good manufacturing practices. [10, 11] An increasing amount of research indicates that traditional medicines have diuretic effects. [12, 13] Clerodendrum indicum (L) Kuntze, a member of the Verbenaceae family, is the plant we have chosen for our investigation. This deciduous shrub is found throughout India's Western Ghats. [14] According to traditional beliefs, this plant's roots may be used as medicine to treat conditions like rheumatism, allergies, body aches, respiratory disorders, infectious diseases, dropsy, eye disorders, fever, inflammation, malaria, opthalmia, rheumatism, snakebite, TB, ulcers, and wounds. [15-17] Several chemical constituents of the plant, such as stigmasterol, bis(2-ethylhexyl) phthalate, hispulidin, serratumin A, acteoside, martynoside, serratumoside-A, myricoside, ursolic acid, spinasterol, spinasteryl-β-D-glucopyranoside, and others, have been found in the aerial, root, and stem parts of the plant. [18-20]

We postulated that the leaves of C. indicum may be useful in treating nephrotoxicity and diuretic conditions based on the literature background. Scientific research on its diuretic and nephroprotective effects in experimentally caused nephrotoxicity generation in laboratory animals is, however, lacking. Therefore, the ethanolic extract of C. was tested against gentamicin-induced nephrotoxicity in Swiss albino rats in the current investigation.

MATERIALS AND METHODS

Collection and authentication of plant material: Leaves of C. indicum were collected from the local area of Meerut (India) in the month of January and authenticated by Department of Botany.

Preparation of ethanolic extract of C. indicum (EECI): The adhering sand and dust particles were scraped off of the C. indicum leaves. With the aid of an electric grinder, it was dried and ground into a coarse powder. Using 95% ethanol as a solvent, 100 g of ground plant material was extracted at 60–70°. To create a viscous mass, the solvent was evaporated at room

temperature. After filtering it, the solvent was eliminated. [21] The brown-colored dried molten mixture was kept in a desiccator until it was needed. To administer the extract orally to animals, sodium carboxymethyl cellulose was used as a suspending agent in distilled water.

Experimental animals: Male Swiss albino rats weighing between 200 and 300 grammes were purchased from a breeder certified by CPCSEA. The animals were housed in six polypropylene cages each, with a 12-hour light and 12-hour dark cycle, and were given free access to commercial pellet food (Hindustan Lever Ltd., Bombay, India) and water at a constant temperature of 25±2°C and a relative humidity of 50±15%. The Institutional Animal Ethics Committee gave its approval to the project. Throughout every experiment, ethical guidelines were closely adhered. [22]

Experimental Design: Five groups of male rats were created: Group V received the conventional medication (Vitamin E 250 mg/kg p.o.) orally for the first 14 days of the trial, while Group I was the control group, Group II was the disease control group, and Group III and IV were treated with EECI at 250 and 500 mg/kg, respectively. Gentamicin was chronically given intraperitoneally (i.p.) to all animals except Group I at a dose of 100 mg/kg body weight. Following the study's conclusion, the kidneys' weight was determined, and several other parameters were noted for each group. [23, 24]

Sampling and biochemical analysis: The second portion of blood was centrifuged at 5000 rpm for 10 minutes. Following the manufacturer's instructions, the acquired clear sera were kept at -20°C until the levels of urea (UR), creatinine (CR), and uric acid (UA) could be measured using colorimetric assay kits from Bayer (Seamon, Germany). [25]

Preparation of renal homogenate: The kidneys were removed and dissected free from the surrounding fat and connective tissue. The renal cortex was isolated and stored at -8°C after each kidney was longitudinally sectioned. The kidney's cortex was then homogenised in a cold potassium phosphate buffer (pH 7.4, 0.05 M). At 4°C, the renal cortical homogenates were centrifuged for 10 minutes at 5000 rpm. [26]

Histopathological examination: Hematoxylin-eosin staining was performed on kidney pieces from each group after they were immediately fixed in 10% neutral formalin for at least 24 hours, dehydrated in graded (50–100%) alcohol, embedded in paraffin, and cut into sections that were 4–5 µm thick. Necrosis, lipid infiltration, fibrosis, lymphocyte infiltration, and other pathological signs of nephrotoxicity were assessed in the sections.

Test for diuretic activity: The animals in group IV received treatment with furosemide at a dose of 4 mg/kg, dissolved in normal saline, and administered intragastrically via gastric canula. Group I was used as a plain control and was given vehicle. Groups II and III received single doses of the ethanol extract of C. indicum at 250 mg/kg and 500 mg/kg body weight, respectively (as in the previous test). Eight hours prior to the drug's administration, food and water were stopped. Following medication, each animal was put in a metabolic cage separately, and the urine that each animal produced over the course of 24 hours was collected in a jar. A "flame photometer" was used to measure the total amount of urine produced and to measure the potassium and sodium concentrations. [27]

Statistical analysis: Using a one-way ANOVA and Dunnet test statistical software, the data were evaluated for statistical significance and compared to the control and acetaminophen-treated groups. A p-value of less than 0.05 was deemed significant. [28]

RESULTS AND DISCUSSION

Effect of EECU on body weights and kidney weights against gentamicin-induced animals: The effects of EECI on body and kidney weights in different groups following gentamicin induction are displayed in Tables 1 and 2. When compared to the disease control group, the data clearly demonstrated that the standard and test groups' body weights had significantly increased. On the other hand, gentamicin induction also caused alterations in kidney weights, but EECI and vitamin E treatment resulted in recovery.

Table 1: Effect of EECI on body weights against gentamicin-induced animal

Groups	Treatment & dose	Before treatment (g)	After treatment (g)
I- Normal	-	223.45±2.37	227.58±2.45
II-Toxic Control	Gentamicin + vehicle 100 mg/kg	221.65±1.65	204.6±3.732**
III-Test 1	EECI 250 mg/kg p.o.	234.65±2.56	229.14±4.67**
IV-Test 2	EECI 500 mg/kg p.o.	232.11±4.76	230.76±3.41***
V-Std. control	Vitamin E 250 mg/kg/p.o.	225.67±2.67	222.62±4.67***

Values are expressed as Mean ± SEM of six animals. Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnett's multiple comparison test." Comparisons were done between (a) Group I versus Group II and (b) Group II versus Group III, IV, and V. **p<0.01, ***p<0.001. ns: Nonsignificant, SEM: Standard error of mean, EECI: Ethanolic extract of C. indicum

Table 2: Effect of EECI on kidney weights against gentamicin-induced animals

Groups	Treatment & dose	Kidney weights (g)
I- Normal	-	0.687±0.003
II-Toxic Control	Gentamicin + vehicle 100 mg/kg	1.423±0.011***
III-Test 1	EECI 250 mg/kg p.o.	0.97±0.005*
IV-Test 2	EECI 500 mg/kg p.o.	0.84±0.006***
V-Standard control	Vitamin E 250 mg/kg/p.o.	0.79±0.023***

Values are expressed as Mean ± SEM of six animals. Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnett's multiple comparison test." Comparisons were done between (a) Group I versus Group II and (b) Group II versus Group III, IV, and V. *p < 0.05, **p<0.01, ***p<0.001. ns: Nonsignificant, SEM: Standard error of mean, EECI: Ethanolic extract of C. indicum

Effect of C. indicum extract on serum urea, uric acid, and creatinine concentrations: When compared to the normal animals, the APAP-treated group's serum creatinine and urea values were considerably higher (**p<0.01), suggesting that severe nephrotoxicity had been induced. Serum urea and creatinine concentrations significantly decreased (**p<0.01, ***p<0.001) in groups III and IV after treatment with the ethanol extract of *C. indicum* as compared to group II, which received gentamycin treatment. In contrast to the control group, the gentamycin-treated groups' uric acid (UA) levels dramatically dropped (**p<0.01). In comparison to the gentamycin-treated group (group II), EECI treatment significantly (***p<0.001) raised the uric acid levels in groups III and IV, respectively. Serum urea, uric acid, and creatinine levels are not significantly affected by the standard medication (group V).

Table 3: Effect of EECI on biochemical parameters

Groups	Urea (mg/dL)	Serum creatine (mg/dL)	Uric acid (mg/dL)
I- Normal	51.00±1.55	1.050±0.032	1.67 ± 0.01
II-Toxic Control	95.11±2.32***	5.53±0.202***	2.45 ± 0.04***
III-Test 1	84.47±1.64*	4.76±0.14***	1.91 ± 0.04**
IV-Test 2	76.48±2.11**	2.12±0.12***	1.84 ± 0.03***
V-Standard control	56.45±2.54***	1.76±0.11***	1.78 ± 0.03***

Values are expressed as Mean ± SEM of six animals. Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnett's multiple comparison test." Comparisons were done between (a) Group I versus Group II and (b) Group II versus Group III, IV, and V. *p < 0.05, **p<0.01, ***p<0.001. ns: Nonsignificant, SEM: Standard error of mean, EECI: Ethanolic extract of C. indicum

Histopathological studies: The histological pattern of a normal kidney, which displays intact glomeruli and Bowman's capsule together with typical tubular brush boundaries, further supported the biochemical data (Figure 1a). Renal tissue treated with gentamycin exhibits significant tubular necrosis and degeneration (Figure 1b). Rats given 250 mg/kg body weight of C. indicum ethanol extract had a typical tubular pattern along with a slight amount of degranulation, oedema, and necrosis (Figure 1c). While the administration of standard vitamin E did not result in any notable alterations in the renal tissues (Figure 1e), treatment with the ethanol extract of C. indicum (500 mg/kg body weight) improved the toxic symptoms in the kidney (Figure 1d).

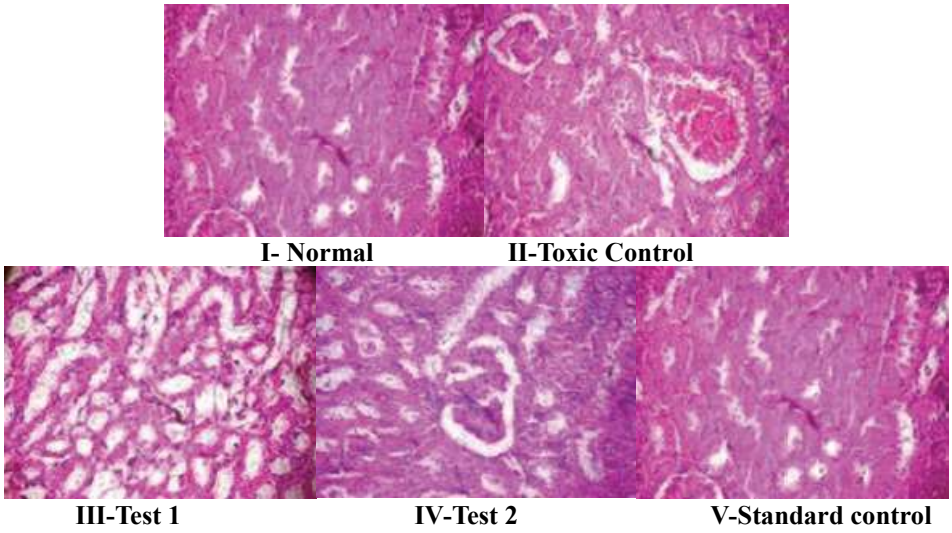

Figure 7. Nephroprotective effect of C. indicum. Histopathological observations (kidney sections stained with Hematoxylin- Eosin, magnification-100×) (a) Normal, (b) toxic control, (c) EECI 250 mg/kg + Gen, (d) EECI 500 mg/kg + Gen, (e) Standard Control.

Diuretic effect:

Total urine output: Urine volume increases significantly (** $p < 0.01$) when 500 mg/kg of C. indicum ethanol extracts are administered in comparison to the control group. It was nearly as much diuresis as that caused by furosemide.

Table 5: Diuretic Activity of Ethanolic Extracts of C. indicum

Groups	Volume of urine (ml /100 gm)				
	After 5 hr	After 10 hr	After 15 hr	After 20 hr	After 24 hr
I- Normal	2.28±0.02	3.43±0.04	3.87±0.02	4.92±0.04	5.11±0.03
II-Test 1	2.23±0.02	4.23±0.02*	4.56±0.03	5.15±0.05	5.56±0.12*
III-Test 2	4.54±0.04	4.52±0.03	4.95±0.02	5.79±0.04	6.34±0.01**
IV-Standard control	3.92±0.02	4.67±0.02	5.15±0.03	5.89±0.04	6.78±0.04**

Values are expressed as Mean ± SEM of six animals. Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnett's multiple comparison test. ***$p<0.001$, ** $p < 0.01$, * $p < 0.05$ with respect to control.

Electrolytes—urinary sodium: It was discovered that C. indicum ethanol extracts considerably (** $p < 0.01$) increased natriuresis. Animals treated with furosemide likewise showed a significant (***$p<0.001$) increase in natriuresis; however, 500 mg/kg had the greatest effect.

Electrolytes—urinary potassium: It has been demonstrated that ethanol extracts considerably raise potassium excretion in urine when compared to the control group (***p0.001, ** p0.01, * p0.05, respectively). The amount of potassium expelled by furosemide was found to be somewhat less than that of the 500 mg/kg extract, despite the fact that it likewise considerably increased potassium output.

Electrolytes—urinary Chloride: When compared to the control group, ethanol extracts have been demonstrated to significantly enhance potassium excretion in urine * $p<0.05$, respectively. The amount of potassium expelled by furosemide was found to be somewhat less than that of the 500 mg/kg extract, despite the fact that it likewise considerably increased potassium output.

Table 4: Effect of EECI on electrolytes

Groups	Sodium	Potassium	Chloride
I- Normal	141.45 ± 2.54	5.65 ± 0.54	91.1 ± 1.43
II-Test 1	151.25 ± 3.43*	5.87 ± 0.32*	95.54 ± 2.26
III-Test 2	147.11 ± 2.11**	5.77 ± 0.77**	94.05 ± 1.44*
IV-Standard control	143.73 ± 0.48***	5.86 ± 0.05***	92.41 ± 2.47***

Values are expressed as Mean ± SEM of six animals. Statistical significance test for comparisons was done by one-way ANOVA, followed by "Dunnett's multiple comparison test. ***p<0.001, ** p < 0.01, * p < 0.05 with respect to control.

DISCUSSION AND CONCLUSION

The goal of the current study was to assess C. indicum's diuretic and nephroprotective properties against gentamycin-induced nephrotoxicity. Therefore, in individuals with normal renal function, gentamicin should be administered at the lowest effective therapeutic doses. An antibiotic called gentamicin is frequently used to treat serious Gram-negative infections. However, its nephrotoxicity limits its clinical application. Free radicals or reactive oxygen metabolites are significant mediators of gentamicin nephrotoxicity, according to multiple lines of evidence [29]. Gentamicin typically builds up in renal proximal tubules and promotes the mitochondria's production of hydrogen peroxide, which is mostly produced by superoxide dismutation [30]. Iron is released from the mitochondria by hydrogen peroxide produced when gentamicin causes oxidative stress in the membranes of the mitochondria. The oxidative stress is accelerated by the liberated iron's combination with gentamicin [31]. Antioxidant medicines have been shown to have the most consistent effects among the primary strategies employed to mitigate or prevent the gentamicin-induced nephrotoxicities [32]. A number of antioxidants, including deferoxamine, methimazole, vitamin E, vitamin C, diethyl dithiocarbamate, L-histidinol, and thymoquinone, have been utilised to lessen the nephrotoxicity caused by gentamicin in rats [33-37].

Rats given gentamicin (toxic control group) experienced acute kidney impairment, as shown by substantial increases in serum creatinine and total protein, as well as a loss in body weight and many histological abnormalities. Serum creatinine, uric acid, and urea levels are considerably reduced after 14 days of treatment with C. indicum at dose levels of 250 mg/kg body weight and 500 mg/kg body weight in comparison to the toxic group.

Both groups gained the same level of significance against the toxic group in the majority of the parameters, including serum creatinine, total protein, kidney weights, and body weights. This indicates that the nephroprotective activity of the C. indicum-treated group and the powerful antioxidant vitamin E-treated group (both groups were compared with the toxic control) was nearly equal. Furthermore, compared to furosemide, it is evident that the EECI has strong diuretic action. In order to identify the active principles causing the actions in our laboratory, more study is being conducted. Accordingly, the assessment of the study concludes that the herbal medication has nephroprotective action and diuretic properties, which have been demonstrated by several animal models and provide numerous connections for further research.

REFERENCES

1. Amit K, Kundan SB, Amteshwar SJ, Richa S. Comparative evaluation of neuroprotective effect of three varieties of Allium cepa in chronic constriction injury induced neuropathic pain. Thai J Pharm Sci 2016;40(1):9-20.
2. Hole RC, Juvekar AR, Roja G, Eapen S, D'Souza SF. 2009. Positive inotropic effect of the leaf extracts of parent and tissue culture plants of Coleus amboinicus on an isolated perfused frog heart preparation. Food Chem 114: 139–141.
3. Holtzman JL. 1995. The role of covalent binding to microsomal proteins in the hepatotoxicity of acetaminophen. Drug Metab Rev. 27:277–97.
4. Kaoua AM, Mahiou-Leddet V, Hutter S, Aïnouddine S, Hassani S, Yahaya I, Azas N, Ollivier, E. 2008. Antimalarial activity of crude extracts from nine African medicinal plants. J Ethnopharmacol 116:74–83.
5. Keaton MR. 1988. Acute renal failure in an alcoholic during therapeutic acetaminophen ingestion. S Med J 81:1163–1166.
6. Kleinknecht C, Broyer M, Gubler M-C, Palcoux J-H. 1980. Irreversible renal failure after indomethacin in steroid-resistant nephrosis. N Engl J Med 302:691.

7. Kumaran A, Karunnakaran RJ. 2007. In vitro antioxidant activities of methanol extracts of Phyllanthus amarus species from India. LWT-Swiss Soc Food Sci Technol 40:344–352.
8. Gummalla P, Anusha VL, Hemalatha CH, Anil KY, Sravani K. Anxiolytic and anticonvulsant activity of methanolic extract of Allium cepa Linn (onion) bulbs in Swiss albino mice. J Pharmacogn Phytochem 2015;4(2):131-5.
9. Patra A., Jha S and murthy P.N: Diuretic activity of different extracts of leaves of Hygrophilaspinosa tanders (acanthaceae), Indian Drugs 2011 48(07): 50-53
10. Bose A., Mondal S., Gupta J.K., Dash G.K and Ghosh T: Studies on diuretic and laxative activity of ethonolic extract and its fraction of cleome rutidosperma aerial parts, Phcog.mag 2006, 2(7): 178-182.
11. Paoulomi C, Aniruddha M, Subhangkar N. Protective effects of the aqueous leaf extract of Aloe barbadensis on gentamicin and cisplatin-induced nephrotoxic rats. Asian Pac J Trop Biomed 2012;2(3):S1754-63.
12. El-Badwi SM, Bakhiet AO, Abdel EH. Hemato-biochemical effects of aqueous extract of Khaya senegalensis stem bark on gentamicin induced nephrotoxicity in Wistar rats. J Biol Sci 2012;12(6):361-6.
13. Harlalka G.V., Patil M.R: protective effect of kalanchoe pinnata (crussulaceae) on gentamicin induced nephrotoxicity in rats, Indian J Pharmacol. 2007 39:201-205.
14. Teklit GA. In vitro antimicrobial efficacy of fractions from onion (Allium cepa) leaves extract from Wukro, Ethiopia. Am J Life Sci 2015;3(5):365-8.
15. Vasiliades J. Reaction of alkaline sodium picrate with creatinine: I. Kinetics and mechanism of formation of the mono-creatinine picric acid complex. Clin Chem 1976;22(10):1664-71.
16. Bibu KJ, Joy AD, Mercey KA. Therapeutic effect of ethanolic extract of Hygrophila spinosa T. Anders on gentamicin-induced nephrotoxicity in rats. Indian J Exp Biol 2010;48(9):911-7.
17. Kusumoto IT, Nakabayashi T, Kida H, Miyashiro H, Hattori M, Namba T, Shimotohno K. 1995. Screening of various plant extracts used in Ayurvedic medicine for inhibitory effects on Human Immunodeficiency Virus Type-1 (HIV-1) protease. Phytother Res 9:180–184.
18. Vishvakrama P, Sharma S. Liposomes: an overview. Journal of Drug Delivery and Therapeutics. 2014 Jun 24:47-55.
19. Vishvakarma P. Design and development of montelukast sodium fast dissolving films for better therapeutic efficacy. Journal of the Chilean Chemical Society. 2018 Jun;63(2):3988-93.
20. Vishvakarma P, Mandal S, Verma A. A review on current aspects of nutraceuticals and dietary supplements. International Journal of Pharma Professional's Research (IJPPR). 2023;14(1):78-91
21. Prabhakar V, Agarwal S, Chauhan R, Sharma S. Fast dissolving tablets: an overview. International Journal of Pharmaceutical Sciences: Review and Research. 2012;16(1):17
22. Vijay KK, Naidu MU, Anwar AS, Ratnakar KS. Probocol protects against gentamicin induced nephrotoxicity in rats. Indian J Pharmacol 2000;32:108-13.
23. Bonkovsky HL, Kane RE, Jones DP, Galinsky RE, Banner B. 1994. Acute hepatic and renal toxicity from low doses of acetaminophen in the absence of alcohol abuse or malnutrition: evidence for increased susceptibility to drug toxicity due to cardiopulmonary and renal insufficiency. Hepatology 19:1141–1148.
24. Chanwitheesuk A, Teerawutgulrag A, Rakariyatham N. 2005. Screening of antioxidant activity and antioxidant compounds of some edible plants of Thailand. Food Chem 92:491–497.

25. Oyeyemi, A. O., Oseni, O. A., Babatunde, A. O., and Molehin, O. R. (2020). Modulatory effect of Polyalthia longifolia leaves against cadmium-induced oxidative stress and hepatotoxicity in rats. J. Compl. Integr. Med. 9. doi:10.1515/jcim-2019-0038
26. Ozaslan, M., and Oguzkan, S. B. (2018). Use of plant extracts in alternative medicine. Pak. J. Biol. Sci. 21 (1), 1–7. doi:10.3923/pjbs.2018.1.7
27. Pais, G. M., Liu, J., Avedissian, S. N., Hiner, D., Xanthos, T., Chalkias, A., et al. (2020). Lack of synergistic nephrotoxicity between vancomycin and piperacillin/tazobactam in a rat model and a confirmatory cellular model. J. Antimicrob. Chemother. 75 (5), 1228–1236. doi:10.1093/jac/dkz563
28. Chattopadhyay RR. 2003. Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: Part II. J Ethpharmcol 89:217–219.
29. Curry Jr RW, Robinson JD, Sughrue MJ. 1982. Acute renal failure after acetaminophen ingestion. J Am Med Assoc 247:1012–1014.
30. Devipriya S, Shyamaladevim CS. 1999. Protective effect of quercetin in cisplatin induced cell injury in the rat kidney. Ind J Pharmacol 31:422–426.
31. El-Beshbishy HA. 2005. Hepatoprotective effect of green tea [Camellia sinensis] extract against tamoxifen-induced liver injury in rats. J Biochem Mol Biol 38:300–306.
32. Favre L, Glasson P, Vallotton MB. 1982. Reversible acute renal failure from combined triamterene and indomethacin: a study in healthy subjects. Ann Intern Med 96:317–320.
33. Gardner CR, Laskin JD, Dambach DM, Sacco M, Durham SK, Bruno MK, Cohen SD, Gordon MK, Gerecke DR, Zhou P, Laskin DL. 2002. Reduced hepatotoxicity of acetaminophen in mice lacking inducible nitric oxide synthase: potential role of tumor necrosis factor-alpha and interleukin-10. Toxicol Appl Pharmacol 184:27–36.
34. Ghosh A, Sil PC. 2007. Anti-oxidative effect of a protein from Cajanus indicus L. against acetaminophen-induced hepato-nephro toxicity. Biochem Mol Biol 40:1039–1045.
35. Harsha VH, Hebbar SS, Hegde GR, Shripathi V. 2002. Ethnomedical knowledge of plants used by Kunabi Tribe of Karnataka in India. Fitoterapia 73:281–287.
36. Vishvakarma P, Mandal S, Pandey J, Bhatt AK, Banerjee VB, Gupta JK. An Analysis Of The Most Recent Trends In Flavoring Herbal Medicines In Today's Market. Journal of Pharmaceutical Negative Results. 2022 Dec 31:9189-98
37. Hernández Canoa J, Volpato G. 2004. Herbal mixtures in the traditional medicine of Eastern Cuba. J Ethnopharmacol 90:293–316.