PROTECTIVE ROLE OF *ZINGIBER OFFICINALE* ON NITROSOMETHYLUREA INDUCED NEPHROTOXICITY ON WISTAR RATS

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Abstract

Kidney is an important organ in the body which is involved in biotransformation of toxins. Kidney disease is a major worldwide problem mainly caused by toxicants. This study was aimed at evaluating the protective roles of Z. officinale on nitrosomethylurea induced kidney injury in Wister rats. Z. officinale was investigated for the presence of phytochemical in ethanolic extract, which showed the presence of terpenoid (18.0 mg/L), alkaloid (21.98 mg/L), saponin (27.64 mg/L), steroid (27.38 mg/L), and flavonoid (22.71 mg/L) while tannin, cardiac glycoside and phenolic were absent. The result from the DPPH radical scavenging activity of the ethanolic extract of Z. officinale showed that when compared to ascorbic acid, the activity of the extract increased with increasing concentration with activity at 42% at 3.125 ug/ml to 78% at 1000 ug/ml. However, the FRAP assay showed a decline with reducing ability with increasing concentration. The kidney biomarkers of urea and creatine showed the clearance of both markers increased in the treated groups when compared to the toxicant groups. The findings from this study shows that Z. officinale was able to protect the kidney from kidney injury induced by NMU.

Keywords; kidney, antioxidant, nitosomethylurea

Introduction

The kidneys are responsible for a number of crucial processes, including low-molecular-weight protein metabolism, excretion of waste products, erythropoietin production, acid-base balance, and electrolyte and volume management. (Madrazo-Ibarra & Vaitla, 2021). Acute kidney injury (AKI) is a frequent condition that has a high risk of in-hospital death(Li et al., 2009). Given the kidney's responsibilities in plasma filtration and the maintenance of metabolic homeostasis, toxic effects on the kidney are connected to medication consumption, and environmental toxicants are both prevalent and expected (Lakshmi & Sudhakar, 2010). The N-nitroso group includes N-nitroso methyl urea (NMU), which can be produced by the body from precursors as well as found in low concentrations in our environment and food. (Verma et al., 2012). The production of antioxidants by the human body serves as one of several defense mechanisms against oxidative or nitrosative effects. Antioxidants are necessary because they protect against oxidative stress and help to prevent chronic illnesses (Kurutas, 2016). The plant's rhizome, called *Zingiber officinale*, is eaten whole as a delicacy, medicinal, or herb. It serves as an appetite stimulant, a narcotic antagonist, and an anti-inflammatory drug, among other medical uses (Zhang et al., 2022). Therefore, the goal of this investigation was to determine how *Z. officinale* protects against Nitrosomethlyurea-induced nephrotoxicity.

Methodology

Preparation of Crude Extract

The *Z. officinale* was collected from Sayedero market, Ilaro Ogun State. Plant Identification was done at the Department of Botany, University of Lagos. Plant Identification no is LUT: 9743. Dirt and sand stains was promptly removed by washing the plant materials followed by peeling and slicing into smaller pieces. The sliced plant was air dried. Dried samples were milled with an electric blender. 400g of the milled rhizome of *Z. officinale* was weighed and soaked in 5 L round bottomed flask containing 2 liters of ethanolic solvent and was sealed with aluminum foil, then kept in shade for 72 hours with occasional shaking of the extracts. The extract was filtered with muslin cloth fabric and concentrated using rotary evaporator at temperature between 40-60^{o}C. The extract was dried at 50°C using a water bath.

Invitro Studies Qualitative and Quantitative phytochemical evaluation

Qualitative and quantitative evaluation of the ethanolic extract of *Z. officinale* was carried out using standard laboratory procedure as earlier described by Ezeonu and Ejikeme (2016).

Invitro Antioxidant activity

The free radical scavenging activity (antioxidant capacity) of the plant extract on the stable radical1,1 -diphenyl-2-picrylhydrazyl (DPPH) was evaluated by the method reported by Aliyu et al. (2013). The total reducing power of the extract was determined according to the method described previously (Ferreira et al., 2007)

Animal Studies

20 Wister rats were weighed between 150g and 200g and it was divided into four groups. All the rats were acclimatized for 7days to enable them adapt to their new environment and they were fed with water and pellet. After their acclimatization, the animal study which lasted for 4weeks.

Group A: (Normal control)

Group B: (Negative control; NMU only 50mg/kg body weight weekly)

Group C: NMU + 200 mg/kg body weight of *Z. officinale* extract was given daily (Salama et al., 2013). Weekly administration of NMU at 50 mg/kg body weight (Sriwiriyajan et al., 2016)

Group D: NMU + 400 mg/kg body weight of *Z. officinale* extract was given daily (Salama et al., 2013). Weekly administration of NMU at 50 mg/kg body weight (Sriwiriyajan et al., 2016)

Biochemical Assays

Estimation of Serum Markers of Kidney function

Animal blood samples were drawn into tubes, allowed to clot, and then centrifuged at 3400 rpm for 10 min at 4°C. Using commercially available Randox kits, serum samples were taken for measuring the kidney indicators urea and creatine. A spectrophotometer was used to measure the markers.

Statistical Analysis

Results were analyzed using graph pad prism software (version 5.0), and the results were expressed as mean ± SD. Samples were experimented in triplicates (n=3), and the results were expressed as mean ± SD and $P<0.05$ were considered significant.

RESULTS

Table 1: Qualitative Phytochemicals of Ethanolic Extract of *Z. officinale*

Phytochemical	Ginger
Terpenoid	+
Alkaloid	+
Saponin	+
Tannin	-
Cardiac glycoside	-
Flavonoid	+
Steroid	+
Phenolics	-

+ = Present; - = Absent

Table 2: Quantitative Phytochemical of Ethanolic Extract of *Z. officinale*

Phytochemicals	Concentration (mg/l)
Steroid	27.38 ± 0.16
Flavonoid	22.71 ±0.78

Alkaloid	21.98 ±0.12
Saponin	27.65 ±0.31
Terpenoid	18.0 ±0.51

Values are expressed as Mean ± SD

Table 3: Showing the DPPH Radical Scavenging Activity of the Ethanolic Extract of *Z. officinale*.

Concentration (µg/ml)	Ginger Activity (%)	Ascorbic Acid Activity (%)
31.25	42.64	97.94
62.5	53.57	98.17
125	58.90	98.27
250	57.79	98.38
500	62.68	98.34
1000	78.23	99.08

Table 4: Showing the Ferric Reducing Activity of the Ethanolic Extract of *Z. officinale*

Concentration (µg/ml)	Ginger Activity (%)	Ascorbic Acid Activity (%)
31.25	64.48	97.38
62.5	63.33	95.79
125	58.75	91.05
250	57.99	82.43
500	56.19	66.66
1000	50.73	31.80

Table 5: Urea and Creatine Concentration of the Kidney of Wister rats induced with NMU & treated with the Ethanolic Extract of *Z. officinale.*

Sample	Urea (Mmol/L)	Creatine (Mmol/L)
Normal control	5.9 ±0.55 [b]	50 ±17 [b]
Negative control	6.7 ±1.40 [a]	53 ±7.6 [a]
Ginger 200 mg/kgbw	6.6 ±0.70	51 ±2.4
Ginger 400 mg/kgbw	5.8 ±0.57[b]	52 ±5.1

Values are expressed as Mean ±SD

[a] Significantly different from Normal control group at $p<0.05$

[b] Significantly different from Negative Control group at $p<0.05$

Discussion

The result (Table 1) reveals the qualitative phytochemical screening of ethanolic extracts of *Z. officinale* showed the present of steroids, alkaloids, terpenoids, saponins and flavonoids but tannins, phenolics and cardiac glycoside were absent. According to Baliga et al. (2013); the chemical constituents present in *Z. officinale* are zingiberene, curcumene, farnesene, zingiberol, D-camphor, Shogaols, Diarylheptanoids, Gingerols, Paradol, Zerumbone, 1-Dehydro-(10) gingerdione, Terpenoids and Ginger flavonoids respectively. Quantitatively from (Table 2) the phytochemicals present were determined to be steroid (27.38 mg/L), flavonoid (22.71 mg/L), alkaloid (21.98 mg/L), saponin (27.64 mg/L) and terpenoid (18.0 mg/L). Ethanolic extract of *Z. officinale* has shown to be rich in steroids, flavonoids and saponin which are known to possess antioxidant activities was reported by Chrubasik et al. (2006).

The DPPH radical scavenging activity of the ethanolic extract of *Z. officinale* showed that in comparison to ascorbic acid, the activity of the extract increased with increasing concentration with activity at 42% at 3.125 ug/ml to 78% at 1000 ug/ml. However, the FRAP assay showed a decline with reducing ability with increasing concentration. This assays is consistently documented with Aliyu et al. (2013). Urea and creatine which are important kidney biomarkers was observed to reduce in the treated groups when compared to the groups untreated showing that the ethanolic extract of *Z. officinale* protected against kidney damage in the treated groups.

Conclusion

In conclusion, the ethanolic extract of *Z. officinale* acts *as a* protective agent on the nephrotoxicity effect of nitrosomethylurea induced Wister rat. The chemical constituents present in the *Z. Officinale* extract allow it to act as an effective medication which possess an antioxidant component during the assessment.

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