© JUN 2026 | IRE Journals | Volume 9 Issue 12 | ISSN: 2456-8880
DOI: https://doi.org/10.64388/IREV9I12-1719355

Mitigatory Roles Of Asystasia Gangetica on Glutathione and Metabolic Enzyme Activities in the Cerebellum of Flunitrazepam-Induced Neurotoxicity in Wistar Rats

GABRIEL EMERSON EBITO[1], BAMIDELE PONLE FAKUNLE[2], ADEJINMI SULIAT OPEYEMI[3]
[1]*Department of Anatomy, Faculty of Basic Medical Sciences, Ekiti State University, Ekiti State, Nigeria*
[2,3]*Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ogun State, Nigeria*

Abstract- ***non-pharmaceutical use of drugs negatively impacts individuals, communities, and global public health. Flunitrazepam (FNZ), a benzodiazepine commonly associated with drug abuse and drug-facilitated sexual assault, exerts its effects by acting on GABA-A receptors. The brain is particularly vulnerable to oxidative damage due to its high oxygen consumption, relatively low antioxidant reserves, and abundance of polyunsaturated lipids. This study evaluated the protective effect of Asystasia gangetica aqueous leaf extract (AGLE) against FNZ-induced neurotoxicity in the cerebellum of Wistar rats. Forty adult male Wistar rats (200–230 g) were randomly allocated into four groups (n = 10 per group): Group 1 (control, distilled water), Group 2 (FNZ, 3 mg/kg bw), Group 3 (FNZ 3 mg/kg bw + AGLE 500 mg/kg bw), and Group 4 (AGLE 500 mg/kg bw). All treatments were administered orally for 21 days. Biochemical parameters assayed in cerebellar homogenates included reduced glutathione (GSH), glutathione peroxidase (GPx), oxidized glutathione (GSSG), GSH/GSSG ratio, glucose-6-phosphate dehydrogenase (G6PD), and lactate dehydrogenase (LDH). FNZ administration caused significant reductions in GSH, GPx, and GSH/GSSG ratio, and significant elevations in GSSG and LDH activity, consistent with oxidative stress and neuronal injury. G6PD activity showed a non-significant decrease. Co-administration of AGLE with FNZ substantially reversed these biochemical alterations toward control values. These findings demonstrate that A. gangetica aqueous leaf extract confers protective effects against FNZ-induced cerebellar oxidative neurotoxicity, and warrants further investigation as a potential adjunct therapeutic agent.***

Keywords: ***Flunitrazepam, Neurotoxicity, Glutathione, Cerebellum, Asystasia Gangetica***

I. INTRODUCTION

Non-pharmaceutical use of drugs poses serious risks to individuals and communities worldwide, contributing to a broad spectrum of socio-medical challenges [1, 2]. Flunitrazepam (FNZ), a potent benzodiazepine marketed under the trade name Rohypnol, is a Schedule IV controlled substance in many jurisdictions and is commonly associated with drug abuse [3, 4]. FNZ is notorious as a drug-facilitated sexual assault (DFSA) agent — often referred to as the 'date rape drug' — because sexual predators exploit its amnestic and sedative properties to incapacitate victims [5–7].

Pharmacologically, FNZ acts as a positive allosteric modulator at the γ-aminobutyric acid type A receptor (GABAA-R), enhancing chloride ion conductance and thereby potentiating inhibitory neurotransmission [8, 9]. While FNZ has legitimate clinical applications — including short-term treatment of severe insomnia and pre-anaesthetic medication — its therapeutic use has been restricted or banned in many countries owing to its abuse potential [5, 6].

The brain is particularly susceptible to oxidative damage because of its exceptionally high oxygen demand, relatively modest antioxidant capacity, and high content of polyunsaturated fatty acids that are prone to lipid peroxidation. Oxidative stress — defined as an imbalance between pro-oxidant reactive oxygen/nitrogen species (ROS/RNS) and cellular antioxidant defenses — is a key mechanism of drug-induced neurotoxicity. Glutathione (GSH), an abundant tripeptide antioxidant comprising cysteine, glutamic acid, and glycine, plays a central role in

neutralizing ROS and maintaining intracellular redox homeostasis in the central nervous system [10].

Asystasia gangetica (L.) T.Anders (Acanthaceae), commonly known as coromandel, Chinese violet, or tropical primrose, is a perennial herb found along roadsides, riverbanks, and forest margins in tropical regions [11]. Its leaves and shoots serve as a food source in parts of Africa and Asia, being rich in proteins, vitamins, carbohydrates, minerals, lipids, and fibers [11]. Phytochemical analyses have identified alkaloids, tannins, glycosides, steroidal saponins, flavonoids, phenols, and terpenoids in the leaves — many of which possess antioxidant, anti-inflammatory, and cytoprotective properties [12]. Despite these promising bioactivities, the neuroprotective potential of A. gangetica against drug-induced cerebellar neurotoxicity has not been systematically investigated.

This study aimed to evaluate the effects of A. gangetica aqueous leaf extract (AGLE) on glutathione status and metabolic enzyme activities in the cerebellum of Wistar rats subjected to repeated FNZ administration. We hypothesized that the antioxidant phytochemicals in AGLE would attenuate FNZ-induced oxidative stress, as reflected by improvements in GSH, GPx, GSSG, GSH/GSSG ratio, G6PD, and LDH activities.

II. MATERIALS AND METHODS

2.1 Plant Collection, Authentication, and Aqueous Extraction

Leaves of A. gangetica were collected from the Ado plant garden, Ekiti State, Nigeria, and authenticated by a taxonomist at the Herbarium, Department of Plant Science and Biotechnology, Ekiti State University (voucher specimen number should be deposited for reproducibility). Leaves were hand-washed with clean water and air-dried at ambient temperature for three weeks. Dried leaves were pulverized using an electric blender. The powder was macerated in distilled water for 48 hours, then filtered through Whatman No. 1 filter paper. The filtrate was concentrated on a rotary evaporator at 50 °C to a semi-solid paste. A stock solution was prepared by dissolving 5 g of paste in 500 mL distilled water, from which an oral dose of 500 mg/kg bw was administered to each rat daily.

2.2 Drug Preparation

Flunitrazepam tablets (Swinol®; Swiss Pharma Nigeria Ltd., Lagos, Nigeria) were dissolved in distilled water at 3 mg/kg bw per rat for oral administration. Tablets dissolve to a blue-tinted solution, which served as a visual confirmation of dissolution.

2.3 Animals and Experimental Design

Ethical approval was obtained from the Institutional Animal Ethics Committee of Olabisi Onabanjo University, Ogun State, Nigeria (reference no.: OOU/SCIENG/EC/0016/130225). All procedures complied with internationally accepted principles for the care and use of laboratory animals.

Forty adult male Wistar rats (200–230 g) were obtained from the animal house of the Department of Anatomy, Olabisi Onabanjo University, Shagamu Campus, and housed in well-ventilated cages under controlled conditions (12 h light/dark cycle, ambient humidity and temperature). Standard commercial pellets and water were provided ad libitum. Animals were acclimatized for one week before the commencement of experiments. Animals were randomly allocated to four groups (n = 10 per group) as shown in Table 1. All treatments were administered once daily by oral gavage for 21 consecutive days.

Table 1. Experimental grouping and treatment protocol.

Group	n	Treatment	Route	Duration (days)	Dose
1	10	Distilled water (control)	Oral	21	—
2	10	Flunitrazepam (FNZ)	Oral	21	3 mg/kg bw

3	10	FNZ + Asystasia gangetica aqueous leaf extract (AGLE)	Oral	21	3 mg/kg bw + 500 mg/kg bw
4	10	Asystasia gangetica aqueous leaf extract (AGLE)	Oral	21	500 mg/kg bw

2.4 Tissue Collection and Biochemical Assays

Twenty-four hours after the last administration, animals were humanely sacrificed by cervical dislocation under light ether anaesthesia. The cerebellum was rapidly dissected, weighed, and homogenized (1:10, w/v) in ice-cold phosphate buffer (0.1 M, pH 7.4) using a glass homogenizer. Homogenates were centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatant was used for all biochemical assays. Reduced glutathione (GSH), glutathione peroxidase (GPx), oxidized glutathione (GSSG), GSH/GSSG ratio, glucose-6-phosphate dehydrogenase (G6PD), and lactate dehydrogenase (LDH) were measured using standard colorimetric assay kits according to the manufacturers' protocols.

2.5 Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using IBM SPSS Statistics version 25. Group differences were assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc multiple comparison test. Statistical significance was set at $p < 0.05$.

III. RESULTS

Biochemical parameters in cerebellar homogenates across the four experimental groups are summarized in Table 2. FNZ administration (Group 2) caused significant reductions in GSH ($p < 0.05$), GPx ($p < 0.05$), and GSH/GSSG ratio ($p < 0.05$), and significant elevations in GSSG ($p < 0.05$) and LDH ($p < 0.05$) compared with the control (Group 1). G6PD activity showed a non-significant decrease in Group 2 relative to Group 1. Co-administration of FNZ and AGLE (Group 3) substantially reversed these changes, with GSH and GPx values returning toward control levels (non-significant vs. Group 1). GSSG and GSH/GSSG remained significantly different from control in Group 3, suggesting partial but incomplete normalization. AGLE alone (Group 4) did not significantly alter any parameter relative to the control.

Table 2. Biochemical enzyme activities (mean ± SEM) in the cerebellum of Wistar rats across experimental groups.

Group	GSH (µmol/mg)	GPx (U/mg)	GSSG (µmol/mg)	GSH/GSSG	G6PD (mU/g)	LDH (U/g)
1 (Control)	0.22 ± 0.03	0.46 ± 0.00	0.04 ± 0.01	6.56 ± 1.66	224.33 ± 4.11	62.79 ± 7.43
2 (FNZ)	0.14 ± 0.01*	0.21 ± 0.02*	0.14 ± 0.01*	1.00 ± 0.04*	208.09 ± 8.11	101.21 ± 1.57*
3 (FNZ+AGLE)	0.20 ± 0.01	0.41 ± 0.03	0.10 ± 0.02*	2.18 ± 0.49*	219.07 ± 11.39	68.39 ± 10.35
4 (AGLE)	0.22 ± 0.01	0.46 ± 0.04	0.04 ± 0.01	5.64 ± 0.85	217.28 ± 9.96	60.74 ± 8.17

* Significantly different from Group 1 (control) at $p < 0.05$ (one-way ANOVA, Tukey post hoc test). FNZ = flunitrazepam; AGLE = A. gangetica aqueous leaf extract; GSH = reduced glutathione; GPx = glutathione peroxidase; GSSG = oxidized glutathione; G6PD = glucose-6-phosphate dehydrogenase; LDH = lactate dehydrogenase.

IV. DISCUSSION

This study investigated the protective capacity of A. gangetica aqueous leaf extract (AGLE) against FNZ-induced cerebellar oxidative neurotoxicity in Wistar rats. Our key findings were: (i) FNZ significantly depleted GSH and GPx activity while elevating GSSG and LDH, indicative of oxidative stress and neuronal membrane damage; (ii) co-administration of AGLE largely reversed these changes; and (iii)

AGLE alone was well tolerated, with no significant alteration of any biochemical parameter compared with control.

4.1 Reduced Glutathione (GSH)

GSH is the most abundant non-protein thiol antioxidant in the brain and serves as a first-line defense against ROS/RNS. When oxidative burden exceeds cellular capacity, GSH is consumed as it reacts with reactive species or is oxidized to GSSG, leading to net GSH depletion and impaired redox homeostasis [10, 13]. In our study, cerebellar GSH was significantly reduced in FNZ-treated rats, consistent with reports of gastric GSH depletion in flunitrazepam-treated Wistar rats [1]. AGLE co-administration restored GSH to near-control levels, likely reflecting the antioxidant phytochemicals (flavonoids, phenols, terpenoids) in the extract, which may spare endogenous GSH by scavenging ROS directly [12].

4.2 Glutathione Peroxidase (GPx)

GPx catalyzes the reduction of hydrogen peroxide and lipid hydroperoxides to water and corresponding alcohols using GSH as a co-substrate, thereby protecting lipid membranes from peroxidative damage. Reduced GPx activity observed in Group 2 is consistent with substrate depletion (reduced GSH availability) or direct enzyme inactivation by ROS. Similar decreases in cerebellar GPx activity following neurotoxin exposure have been reported in cocaine-treated rats [14], supporting a common oxidative mechanism across different substances of abuse. AGLE co-administration significantly restored GPx activity, corroborating its antioxidant efficacy.

4.3 Oxidized Glutathione (GSSG) and GSH/GSSG Ratio

The GSH/GSSG ratio is widely regarded as a sensitive index of intracellular redox status. FNZ caused a significant increase in GSSG and a concomitant decrease in the GSH/GSSG ratio, indicating a pronounced shift toward an oxidized intracellular environment. This pattern is consistent with findings in cadmium-induced neurotoxicity [15] and cocaine-induced cerebellar oxidative stress [14], in which decreased GSH/GSSG ratios were associated with neuronal injury. Notably, the GSH/GSSG ratio in Group 3 (FNZ + AGLE) did not fully return to control levels, suggesting that while AGLE provides substantial protection, the 500 mg/kg bw dose may not be sufficient for complete normalization, or that some degree of ongoing oxidative stress persists during co-administration of FNZ.

4.4 Glucose-6-Phosphate Dehydrogenase (G6PD)

G6PD catalyzes the rate-limiting step of the pentose phosphate pathway, generating NADPH, which is indispensable for the regeneration of GSH from GSSG by glutathione reductase. Although G6PD activity showed a non-significant trend toward reduction in FNZ-treated rats, the observation that AGLE partially restored G6PD suggests that maintenance of NADPH supply may contribute to the extract's protective mechanism. Future studies should include measurements of NADPH levels and glutathione reductase activity to fully characterize this pathway.

4.5 Lactate Dehydrogenase (LDH)

LDH is a cytosolic enzyme released into the extracellular compartment upon loss of plasma membrane integrity, and is therefore widely used as a biomarker of cytotoxicity. The significant elevation of LDH in FNZ-treated rats indicates neuronal or glial cell damage, likely secondary to oxidative stress-induced membrane disruption. Reversal of LDH elevation by AGLE co-administration supports a membrane-stabilizing or cytoprotective effect of the extract, possibly mediated by inhibition of lipid peroxidation.

4.6 Limitations

Several limitations of this study merit acknowledgment. First, the study used only one dose of FNZ and one dose of AGLE; dose-response relationships were not characterized. Second, only male rats were used, limiting generalizability across sexes. Third, while biochemical endpoints were comprehensively assessed, histological evaluation of cerebellar tissue was not performed, which would have provided morphological corroboration of the biochemical findings. Fourth, the phytochemical composition of the AGLE batch used was not quantified, making it difficult to attribute protective

effects to specific bioactive compounds. Finally, the mechanism of neuroprotection (e.g., direct ROS scavenging versus upregulation of endogenous antioxidant pathways) was not directly examined. Future studies should address these gaps.

V. CONCLUSION

Repeated oral administration of flunitrazepam (3 mg/kg bw for 21 days) produced significant oxidative stress in the rat cerebellum, evidenced by depletion of GSH and GPx, elevation of GSSG and LDH, and a markedly reduced GSH/GSSG ratio. Co-administration of A. gangetica aqueous leaf extract (500 mg/kg bw) substantially reversed these biochemical alterations, demonstrating significant neuroprotective activity. These findings support the potential of A. gangetica as a complementary strategy against FNZ-induced cerebellar neurotoxicity and encourage further investigation into its active constituents, optimal dosing, and mechanisms of action.

VI. ACKNOWLEDGMENT

The authors appreciate all colleagues and technical staff who contributed to the successful execution of this study. The authors also acknowledge the Ethical Committee of Olabisi Onabanjo University for institutional approval.

REFERENCES

[1] Akhigbe RE, Oluwole DT, Adegoke TE, Hamed MA, Anyogu DC, Ajayi AF. Suppression of glutathione system and upregulation of caspase 3-dependent apoptosis mediate rohypnol-induced gastric injury. Redox Rep. 2022;27(1):111–118. doi:10.1080/13510002.2022.2074128

[2] Ajayi AF, Oluwole DT, Akhigbe RE, Hamed MA, Ajayi LO. Proton pump dysfunction and upregulation of caspase-3 activity via oxidative-sensitive signaling mediate rohypnol-induced testicular toxicity. Andrologia. 2023;2023:7215328. doi:10.1155/2023/7215328

[3] Eze GI, Akonoafua KA. Effects of oral ingestion of flunitrazepam and alcohol mixture on the cerebellum of the adult Wistar rat. NISEB J. 2019;19(3).

[4] Udodi PS, Ezejindu DN. A study on the neurotoxicity of flunitrazepam (Rohypnol) administration on the cerebral cortex of adult Wistar rats. Adv Pharmacol Pharm. 2021;9(2):26–32. doi:10.13189/app.2021.090202

[5] Ohshima T. A case of drug-facilitated sexual assault by the use of flunitrazepam. J Clin Forensic Med. 2006;13(1):44–45. doi:10.1016/j.jcfm.2005.05.006

[6] Turina AV, Perillo MA, et al. Flunitrazepam–membrane binding. In: Neuropathology of Drug Addictions and Substance Misuse. 2016.

[7] United States Drug Enforcement Administration. Drug Fact Sheet: Rohypnol. October 2022. Available at: https://www.dea.gov/factsheets/rohypnol

[8] Griffin CE, Kaye AM, Bueno FR, Kaye AD. Benzodiazepine pharmacology and central nervous system-mediated effects. Ochsner J. 2013;13(2):214–223.

[9] National Center for Biotechnology Information. PubChem Compound Summary for CID 3380, Flunitrazepam [Internet]. 2025. Available at: https://pubchem.ncbi.nlm.nih.gov/compound/Flunitrazepam

[10] Aoyama K. Glutathione in the brain. Int J Mol Sci. 2021;22(9):5010. doi:10.3390/ijms22095010

[11] Kumar KA, Umamaheswari M, Sivasanmugam AT, Subhadradevi V, Somanathan SS, Ravi T. Protective effect of Asystasia gangetica on oxidative damage in the small intestine of streptozotocin-induced diabetic rats. Orient Pharm Exp Med. 2009;9(4):307–314.

[12] Uroko RI, Amarachi A, Nweje-Anyalowu PC, Uko OE, Abuachi PT. Effects of Asystasia gangetica extract on biochemical parameters and liver histomorphology of monosodium glutamate-induced rats. Plant Biotechnol Persa. 2021;3(2):1–10.

[13] Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biol Chem. 2003;384(4):505–516. doi:10.1515/BC.2003.059

[14] López-Pedrajas R, Ramírez-Lamelas DT, Muriach B, et al. Cocaine promotes oxidative stress and microglial-macrophage activation in rat cerebellum. Front Cell Neurosci. 2015;9:279. doi:10.3389/fncel.2015.00279

[15] Shukla GS, Srivastava RS, Chandra SV. Glutathione status and cadmium neurotoxicity: studies in discrete brain regions of growing rats. Fundam Appl Toxicol. 1988;11(2):229–235. doi:10.1016/0272-0590(88)90147-9

[16] Halliwell B. Oxidative stress and neurodegeneration: where are we now? J Neurochem. 2006;97(6):1634–1658. doi:10.1111/j.1471-4159.2006.03907.x

[17] Pei J, Pan X, Wei G, Hua Y. Research progress of glutathione peroxidase family (GPX) in redoxidation. Front Pharmacol. 2023;14:1147414. doi:10.3389/fphar.2023.1147414

[18] Chen PH, Tjong WY, Yang HC, et al. Glucose-6-phosphate dehydrogenase, redox homeostasis and embryogenesis. Int J Mol Sci. 2022;23(4):2017. doi:10.3390/ijms23042017

[19] Meng Q, Zhang Y, Hao S, et al. Recent findings in the regulation of G6PD and its role in diseases. Front Pharmacol. 2022;13:932154. doi:10.3389/fphar.2022.932154

[20] Dave A, Maru L, Jain A. LDH (lactate dehydrogenase): a biochemical marker for the prediction of adverse outcomes in pre-eclampsia and eclampsia. J Obstet Gynaecol India. 2016;66(1):23–29. doi:10.1007/s13224-014-0645-x