



Antinutrient Composition and Effect of Ethanol Extract of *Vernonia Amygdalina* Stem on Serum Lipid Profile in Monosodium Glutamate-Intoxicated Rats

Journal Homepage: www.jist.org.in; Email: article@jist.org.in



¹Akachukwu D, ¹Egbonu A.C.C., ¹Ibeh, C.E.

¹Department of Biochemistry, Michael Okpara University of Agriculture, Umudike



ARTICLE INFO

#Corresponding author: Department of Biochemistry, Michael Okpara University of Agriculture, Umudike e-mail-dorisakachukwu@yahoo.com

How to cite this article: Akachukwu D, Egbonu ACC, Ibeh CE. Antinutrient Composition and Effect of Ethanol Extract of *Vernonia Amygdalina* Stem on Serum Lipid Profile in Monosodium Glutamate-Intoxicated Rats. *J Ind. Soc. Toxicol* 2020;16:2. 9-13. DOI: [jist.org.in/10.31736/2020v16i2/9-13](https://doi.org/10.31736/2020v16i2/9-13).

Keywords:

Antinutrient; hyperlipidemia; lipid profile; monosodium glutamate; *Vernonia amygdalina*

Conflicts of Interest and funding: Declared none

Received – 08 December 2020

Accepted – 28 January 2021

Published – 31 January 2021

©2019 The Journal of Indian Society of Toxicology (JIST), Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Pondicherry, 60 5006, India. Further help related to Publication & submission: Dr. A. P. Patra, Editor, Email: rambika_editor@jist.org.in And, Subscription & payment related queries at: Dr. V. V. Pillay, Treasurer, Email: toxicology@aims.amrita.edu

INTRODUCTION

Hyperlipidemia is a key risk factor that contributes to development and severity of cardiovascular diseases globally^[1]. It was strongly associated with high concentration of serum total cholesterol, low density lipoprotein, very low density lipoprotein, reduced high density lipoprotein, altered redox homeostasis.^[1,2,3] In particular, it was noted that increased release of free

ABSTRACT

Vernonia amygdalina stem as food waste was assessed for antinutrients and its effect on serum lipid profile of monosodium glutamate-intoxicated rats. Twenty (20) adult male Wistar rats were randomly assigned into five groups (A-E). Group A (control) rats were administered water only. Group B rats received 8000 mg/kg body weight monosodium glutamate (MSG). Group C rats received the sample extract while those in groups D and E received 8000 mg/kg body weight MSG plus 200 mg/kg and 400 mg/kg body weight extract, respectively. Antinutrients detected were: tannin (130.06 ± 11.67 mg/100 g), trypsin inhibitor (7.75 ± 0.02 µl/mg) and phytate (1.10 ± 0.01 mg/100 g). After 14 days exposure, triacylglycerol (TAG) concentration in the MSG-rat group was significantly ($p < 0.05$) higher as compared to that in the other rat groups except the group E rats. Total cholesterol (TCHOL) and low-density lipoprotein (LDL) concentration in the MSG-treated rats were significantly ($p < 0.05$) higher than the corresponding parameters in the other rat groups. Rats in the MSG group had significantly ($p < 0.05$) lower high density lipoprotein (HDL) concentration compared to the low and high dose extract groups (D and E). Thus tannin is the most abundant out of the determined antinutrients in *Vernonia amygdalina* stem. And, the sample extract showed a significant lipid lowering effect either alone or simultaneously with MSG-intoxication. The apparently synergistic interactive effect of the sample extract with MSG-intoxication on the HDL concentration in the rats could be significant hence warrants follow-up.

radicals induces oxidative stress, atherosclerosis and enhances lipid peroxidation.^[3] Over the years, bioactive principles contained in most plants have been exploited in the management of various diseases.^[4] Such plant-based bioactive principles include antinutrients principally used by plants for defence against predatory attacks and

for combating diseases.^[4] Essentially, different plant stem and roots serve as chewing stick for oral health. One example of such plant, *Vernonia amygdalina*, belongs to the family of *Compositae*. *Vernonia amygdalina* is predominant in tropical grasslands. It is a shrub of about 5 m high with white fragrant flowers that occur in panicles. It closely resembles, but quite different from, *Vernonia colorata* that has hairy leaves.^[5] The *Vernonia amygdalina* leaves serve as vegetables for soup preparation in Nigeria where it is commonly known as 'bitter leaf' or 'Shiwaka' in Hausa, 'Olugbu' in Igbo and 'Ewurojije' in Yoruba. The bitter taste of *Vernonia amygdalina* plant parts has been attributed to the presence of some antinutrients like saponin, tannins and glycosides.^[6] Some of these antinutrients reportedly possess anticorrosive^[7] and antifungal properties^[8] while tannins and phytates particularly bind to and consequently precipitate organic compounds (amino acids, proteins), irons as well as phytochemicals including alkaloids.^[9] Thus, presence of some of these antinutrients could reduce nutrients bioavailability. This study therefore aimed to determine the antinutrients in *Vernonia amygdalina* stem - a food waste and effect of its ethanol extract on the serum lipid profile of normal and monosodium glutamate-intoxicated rats. To achieve the aim, respective content of tannins, trypsin inhibitor and phytates was assessed as anti-nutrients while the concentration of triglycerol (TAG), total cholesterol (TCHOL), high density lipoprotein (HDL), low density lipoprotein (LDL) as well as their relevant corresponding ratios were determined to evaluate the effect of *Vernonia amygdalina* stem extract on normal and monosodium glutamate intoxicated rats.

MATERIALS AND METHODS

Collection, Identification, Preparation and Extraction of Plant Material

Fresh stem of *Vernonia amygdalina* were harvested from a farm in Amawom Oboro, Ikwuano LGA of Abia State, Nigeria. The plant material was authenticated by Prof H.O Edeoga of the Department of Plant Science and Biotechnology, Micheal Okpara University of Agriculture, Umudike, Abia State, Nigeria. The leaves were removed from the stem and the stem was air-dried for 14 days and later ground to powder using a grinding machine.

Cold maceration method was used for the extraction.

Briefly, two hundred grammes (200 g) of the ground stem powder were soaked in 1000 ml absolute ethanol in a covered amber bottle that was shaken at intervals for 72 hrs. Whatman No 1 filter paper was used to filter the solution and the filtrate obtained was evaporated to dryness using a water bath (DK-420) at 50°C, and thereafter stored in the refrigerator until used.

Determination of Antinutritional Factor in Vernonia Amygdalina Stem

Tannins concentration was determined by Folin-Dennis colorimetric method as described by Pearson.^[10] The method described by Russell^[11] was employed for phytate determination while trypsin inhibitor was by the method described by Prokopet and Unlenbruck.^[12]

Animal Study

Toxicity was induced using 8000 mg/kg body weight of the monosodium glutamate (MSG) (dissolved in water) which was orally administered to the rats daily for 14 days according to Mariyamma *et al.*^[13] Previous studies by Egbuonu and Oriji^[14] and Egbuonu and Ekwuribe^[15] indicated that MSG (8000 mg kg⁻¹ B.W) could alter serum lipid parameters and induce stress in rats. Twenty (20) male albino rats (100- 160 g; 8 weeks) used in this study were obtained from the animal breeding unit of the College of Veterinary Medicine, University of Nigeria, Nsukka. They were acclimatized for one week and randomly distributed into 5 groups (A-E) of 4 rats each. Extract was administered to the rats according to body weight ratio as shown below:

- Group A (control) : Distilled water (2 ml kg⁻¹ B.W).
- Group B : MSG (8000 mg kg⁻¹ B.W)
- Group C : Extract (300 mg kg⁻¹)
- Group D : MSG (8000 mg kg⁻¹) +
Extract (200 mg kg⁻¹ B.W)
- Group E : MSG (8000 mg kg⁻¹) +
EXTRACT (400 mg kg⁻¹ B.W)

After two weeks of oral administration, the rats were sacrificed after an overnight fast by cervical puncture and the blood was collected into clean plain bottles, allowed to clot, centrifuged at 3000 rpm for 5 minutes. The resultant serum was collected and used for biochemical analysis. The ethical guidelines for handling laboratory animals of National Institute of Health, USA as approved by the College of Veterinary Medicine Ethical Committee of Michael Okpara University of Agriculture Umudike,

Nigeria was duly followed.

Biochemical Analysis

Serum total cholesterol concentration was determined using the method of Allain *et al.*^[16] as contained in QCA commercial kits. Serum triglyceride concentration was determined using the method of Albers *et al.*^[17] as contained in Randox commercial kit. Serum HDL-cholesterol concentration was determined using the method of Albers *et al.*^[17] as contained in QCA commercial kits.

Statistical Analysis

Data obtained were subjected to one way analysis of variance to test for difference among all the groups. Duncan's multiple range tests was used to test for significant differences among the means and p – value of < 0.05 was considered statistically significant. Result values were expressed as mean \pm SEM.

RESULTS AND DISCUSSION

Table 1 presents the antinutrient content of the ethanol extract of *Vernonia amygdalina* stem. It reveals that the sample extract contains (in increasing order) phytate (1.10 ± 0.01 mg/100 g), trypsin inhibitor (7.75 ± 0.02 μ l/mg) and tannin (130 ± 11.67 mg/100 g). Interestingly, tannin content (mg/100 g) obtained in this study is markedly higher than 0.0557 mg/100 g and 50.70 ± 0.39 mg/100 g reported by Oko *et al.*^[18] and Nimenibo-Uadia *et al.*^[19] respectively for *V. amygdalina* stem, which could be attributed to seasonal and location variation. Tannin exhibited antioxidant and antidiabetic properties^[20]. Generally, high tannin content in foods could be deleterious to health as they form complexes with, and limit the bioavailability of proteins and other biomolecules including carbohydrates, pectin, hemicelluloses and divalent metals. However, simple processing methods like boiling could be employed to reduce tannin content^[21], hence recommend in order to harness the rich tannin content of the *V. amygdalina* stem noted in the present study.

Hyperlipidemia is a key predisposing factor to development of atherosclerosis which is a threat to the development of coronary heart diseases including stroke and myocardial infarction^[22]. Results from this study showed that the triacylglycerols (TAG), total cholesterol (TCHOL) and low density lipoproteins (LDL) of the

monosodium glutamate (MSG) group were higher ($p < 0.05$) while the HDL was lower ($p < 0.05$) than the other groups indicating, possible alteration in cholesterol metabolism. This agrees with the report of Okediran *et al.*^[23] and Egbonu and Ekwuribe^[15] who reported hyperlipidemia in rats following MSG administration at 8000 mg kg^{-1} body weight. Administration of *Vernonia amygdalina* stem extract led to reduced ($p < 0.05$) TAG, TCHOL and LDL in the rats. Monosodium-intoxicated rats co-treated with 200 mg kg^{-1} body weight of extract had significantly lower ($p < 0.05$) TAG, TCHOL and LDL, suggesting possible ameliorative effect of *Vernonia amygdalina* stem extract in MSG-induced hyperlipidemia in the rats. Triacylglycerol (TAG) reduction by the extract could be by activation of lipoprotein lipase, an endothelium bound enzyme that hydrolyses triacylglycerol into fatty acids, consequently, resulting in the noted decreased concentration as reported by Sikarwar and Patil.^[24] Similarly, the extract in this study, could have reduced TCHOL in the rats by stimulating cholesterol-7-alpha-hydroxylase and/or decreasing hepatic HMG CoA reductase activity that convert cholesterol to bile acids. These enzymes were not determined in the present study to confirm the suggestions and possible mechanisms thereto, hence further studies in that direction are warranted and recommended.

Low density lipoproteins transport (LDL) transports 60-70% cholesterol to body cells. Its concentration usually increases as total cholesterol increases. In this study, the extract could have possibly enhanced liver cells ability to discharge LDL from blood by increasing the LDL receptors density that binds to apolipoprotein B.^[25] High density lipoproteins (HDL) scavenge excess cholesterol and its esters in the blood and peripheral tissues, transports them to the liver where they are broken down to bile acids. It is termed protective cholesterol because of its ability to reduce blood and peripheral tissue concentrations and prevention of plaque formation that block the aorta.^[26] Results of this study showed increased ($p < 0.05$) HDL concentration in the rats by the sample extract. This could be by increased activity of lecithin-cholesterol acyl transferase that incorporates free cholesterol into HDL,^[27] warranting further confirmatory studies.

Ratios of components of lipid profile are used as markers to evaluate development of atherosclerosis,^[28] as well as extent of coronary heart diseases. Rats in the extract group had lower ($p < 0.05$) CHOL: TAG but higher TAG/TCHOL ratio compared to the MSG group. Also, MSG

group had lower ($p < 0.05$) HDL/LDL and LDL/HDL ratios compared to other groups. In a similar study, Egbuonu and Ekwuribe^[1] reported high TCHOL:TAG ratio and low TAG:TCHOL ratio in MSG-intoxicated rats administered *Mangifera indica seed* kernel extract while rats in the MSG-intoxicated group had high HDL:LDL and low LDL:HDL ratios. An indepth biochemical implication of lipid profile ratios has not been agreed upon, though Ojiakor and Nwanjo^[29] suggested that a greater than 0.3 HDL/TCHOL ratio and a less than 2.3 LDL/HDL ratio suggest low risk of peripheral arterial disease. Results from this study suggest that the extract could attenuate the hyperlipidemic effects induced by MSG, thereby eliciting beneficial effects by lowering the triacylglycerol, total cholesterol, and LDL while increasing the HDL level in the rats.

CONCLUSION

In conclusion, tannin is the most abundant out of the determined anti-nutrient in *Vernonia amygdalina* stem. And, the sample extract showed significant lipid lowering effect either alone or simultaneously with MSG intoxication. The apparently synergistic interactive effect of the sample extract with MSG intoxication on the HDL concentration of rats may be significant and warranting follow-up.

Limitations of the Study

The study was only conducted using male albino Wistar rats.

Acknowledgements

The authors appreciate the space and equipment provided by laboratory staff of Department of Biochemistry.

REFERENCES

- WHO. Cardiovascular disease (CVDs) Factsheet. Available from: <http://www.who.int/mediacentre/factsheets/fs317/en>.
- Rerkasen K, Gallagher PJ, Grimble RF, Calder PC, Shearman CP. Managing hypercholesterolemia and its correlation with carotid plaque morphology in patients undergoing carotid endarterectomy. *Vasc Health Risk Manag*. 2008; 4:1259–64.
- Hannan PA, Khan JA, Ullah I, et al. Synergistic combinatorial antihyperlipidemic study of selected natural antioxidants; modulatory effects on lipid profile and endogenous antioxidants. *Lipids Health Dis*. 2016; 15: 151.
- Anibijuwon II, Oladejo BO, Adetitun DO, Kolawole OM. Antimicrobial activated of *vernoniaamygdalina* against oral microbes. *Global J Pharm*. 2012; 6(3): 178-85.
- Iwu A, Kokowara JO. Medicinal Plants of east Africa. Africa publish house, 1996; 1-7.
- Arhoghro EM, Ekpo KE, Anosike EO, Ibeh GO. Effect of aqueous extract of bitter leaf (*Vernoniaamygdalina*Del.) on carbon tetrachloride induced liver damage in albino wistar rats. *Eur J Scient.Res*, 2009; 26: 122-30.
- Odiongenyi AO, Odoemelum SA, Eddy NO. Corrosion inhibition and adsorption properties of ethanol extract of *Vernoniaamygdalina* for the corrosion of mild steel in H₂SO₄. *Port. Electrochim Acta*. 2009; 27: 33-45.
- Nduagu C, Ekefan EJ, Nwankiti AO. Effect of some crude plant extracts on growth of *Colletotrichumcapsici*(Synd) Butler and Bisby, causal agent of pepper anthracnose. *J Appl .Biosci*. 2008; 6: 184-90.
- Gemedede HF, Negussie R. Antinutritional factors in plant foods: potential benefits and adverse effects. *Inter J Nutr Food Sci*. 2014; 3: 284-289.
- Pearson D. Chemical Analysis of Food. Churchill Living Stone Edinburgh, UK; 1976.
- Russel HS. India-New England Before the May Flower: University Press of New England Handover; 1980.
- Prokopet G, Unlenbruck KW. Protectineeineneneksalse anti-kowperahlichverbindungen dish. *Ge .Heit*, 2002; 23: 318.
- Mariyamma T, Sujatha KS, Sisilamma G. Protective effect of *Piper longum* (Linn.) on monosodium glutamate induced oxidative stress in rats. *IJEB*. 2009; 47: 186-92.
- Egbuonu ACC, Oriji SO. Pulverized *Mangifera indica* (mango) seed kernel mitigated monosodium glutamate-intoxicated rats' kidney histology and biofunction. *J Nutr Health Food Sci*. 2017; 5: 1-7.
- Egbuonu ACC, Ekwuribe GA. Pulverized *Mangifera indica* (Mango) seed-kernel modulated serum lipid profile in monosodium glutamate-challenged rats. *J Appl. Biotech*. 2017; 5: 72-87.
- Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem*. 1974; 20: 470.
- Albers JJ, Cheung MC, Hazzard WR. High density lipoproteins in myocardial infarction survivors. *Metabolism*. 1978; 27: 479.
- Oko OJ, Odoh R, Tongu SM, Anidobu CO, Udegbunam IS, Umar H. Alkaloid, tannin profiles and mineral element composition of the leaves and stem of vernonia amygdalina (bitter leaf) plant harvested from wukari town, taraba state, north-east Nigeria. *Eur J Pure Appl Chem*. 2018; 5: 1-11.
- Nimenibo-Uadia R, Ugwu I, Erameh T, Osunde E. Estimation of tannins, alkaloids, saponins and proximate composition of Vernonia amygdalina (Del) root. *Int J Herb Med*. 2017; 5: 88-92
- Yokozawa T, Cho EJ, Park CH, Kim JH. Protective effect of proanthocyanidin against diabetic oxidative stress. *eCAM*. 2012; 623879.
- Nzewi DC, Egbuonu ACC. Effect of boiling and roasting on some antinutrient factors of asparagus bean (*Vignasesquipedalis*) flour. *Afr J Food Sci Tech*. 2011; 2: 075-078.
- Vaziri ND, Norris K. Lipid disorders and their relevance to outcomes in chronic kidney disease. *Blood Purif*. 2011; 31:189–96.
- Okediran BS, Olurotimi AE, Rahman SA, Michael OG, Olukunle JO. Alteration in the lipid profile and liver enzymes of rats treated with monosodium. glutamate. *Sokoto J Vet Sci*. 2014; 12: 42-46.
- Sikarwar MS, Patil MB. Antihyperlipidemic effect of ethanolic extract of *Hibiscus rosasinensis* flowers in hyperlipidemic rats. *J Pharm Sci*. 2011; 1: 117–122.
- Baum JA, Teng H, Erdman JW, et al. Long-term intake of soy protein improves blood lipid profiles and increases mononuclear cell low-density-lipoprotein receptor messenger RNA in hypercholesterolemic, postmenopausal women. *Am J ClinNutr*. 1998; 58: 545.
- Kim HP, Park H, Son KH, Chang HW, Kang SS. Biochemical pharmacology of bioflavonoids: Implication in the anti-inflammatory action. *Arch Pharm Res*. 2008; 31: 265–73.
- Geetha G, Kalavalarasariel GP, Sankar V. Antidiabetic effect of *Achyranthesrubrofusca* leaf extracts on alloxan induced diabetic rats. *Pak J Pharm Sci*. 2011; 24: 193–199.
- Kastelein JJP, van der Steeg WA., Holme I, et al. Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation*. 2008; 117: 3002–3009.
- Ojiakor A, Nwanjo H. Effect of vitamin E and C on exercise induced oxidative stress. *Global J Pure Appl Sci*. 2006; 12: 199–202.