ORIGINAL ARTICLE

Evaluation of atrazine toxicity and the protective effect of Vernonia Amygdalina on Haematological, Biochemical, and Histopathological indices in Guinea Pigs (Cavia porcellus)

[1]*Akinyemi,I. G., [2]Jarikre,T.A., [3] Omonona, A.O, [4] Emikpe, B. O.

[1] Forestry Research Institute of Nigeria, P.M.B. 5054, Jericho Hill, Ibadan, Oyo State, Nigeria.
[2] Department of Veterinary Pathology, University of Ibadan
[3] Department of Wildlife and Ecotourism Management, University of Ibadan
[4] School of Veterinary Medicine, KNUST

ABSTRACT

Pesticides can induce toxicity in both biodiversity and humans, and unregulated pesticide application can exacerbate environmental pollution, contribute to wildlife habitat degradation, and accelerate species decline. This study investigated the haematological, biochemical, and histopathological effects of atrazine exposure in guinea pigs (Cavia porcellus) and assessed the ameliorative potential of aqueous bitter leaf extract (Vernonia amygdalina). Thirty guinea pigs were assigned to six groups and treated for 8 weeks with atrazine at 1,500 or 3,000 mg/kg body weight (BW), administered alone or in combination with aqueous bitter leaf extract, alongside control and extract-only groups. Atrazine exposure induced mild anemia, as evidenced by reduced packed cell volume, haemoglobin concentration, and red blood cell count, and increased serum creatinine, blood urea nitrogen, bilirubin, cholesterol, malondialdehyde (MDA), and hydrogen peroxide (H_2O_2). Liver function markers (AST, ALT, and ALP) were evaluated but did not differ significantly among the groups. Atrazine also altered antioxidant defence, including reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione-S-transferase (GST). Histopathological evaluation revealed dose-dependent hepatic, pulmonary, cardiac, and splenic lesions, which were markedly ameliorated by aqueous bitter leaf extract. Treatment with aqueous bitter leaf extract restored haematological parameters, reduced oxidative stress, improved biochemical alterations, and mitigated tissue damage. This study underscores the potential of V. amygdalina as a natural protective agent against atrazine toxicity and provides insights into its possible applications for managing pesticide exposure in the environment.

Keywords: Oxidative stress, Vernonia amygdalina, Cavia porcellus, and antioxidant

*Corresponding Author
AKINYEMI,I.G,Department of Wildlife and Ecotourism, Forestry Research Institute of Nigeria, PMB 5054, Jericho, Ibadan
idloveukpe@yahoo.co.uk; +234 703 301 9174
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INTRODUCTION

Pesticides, encompassing a broad spectrum of chemical compounds, are widely utilised to control unwanted plants, animals, and pests, serving as crop protectants, defoliants, desiccants, and fruit thinning agents, while also helping preserve harvested crops during storage and transportation [1] These chemicals are designed to inhibit or eliminate various pests including insects, rodents, fungi, nematodes, mollusca, weeds, plant growth regulators and other compounds that, if inadvertently ingested, can pose toxic risks to humans [2, 3]. Also, in the views of [4] pesticides could either be compounds synthesized naturally or chemically for the control of varieties of pests. The researchers further stressed their usages in a variety of sectors including food, forestry, agriculture, aquaculture to mention but a few. While pesticides are intentionally used to control pests, they may also harm non-target species, including humans, with residual traces often detected in the environment following application [5]. The pesticides likewise exhibit their toxicity in living systems [4]

These residues, prevalent in soils, water, and crops, can persist and bioaccumulate within the food chain, exposing humans and animals to potentially harmful concentrations through food and water consumption. Residual pesticide traces have been detected in a variety of foods, including meat, milk, fish, fruits and vegetables, contributing to health risks for populations who consume these products or are exposed to pesticide-treated crops [6].

Research suggests that pesticides can induce toxicity in both biodiversity and humans, as observed by [7] and occasional, unregulated pesticide applications can exacerbate environmental pollution, contribute to wildlife habitat degradation and accelerate species decline [8]. The human body is exposed to pesticides either directly or indirectly and the severity depends on exposure time and level of concentration [4]. Although their release from the body could be excreted through the urinary, biliary and secretory gland, long-term accumulation increases the concentration of toxins inside the body organs and cause chronic diseases such as neurotoxicity, cancer, necrosis, asthma, reproductive disorder, cardiac disease, diabetes, etc. [9]. Pesticides are notable for either mimicking or delaying the release of natural hormones thereby disrupting endocrine function with a resultant decreased fertility, neurological or behavioural dysfunctions, thyroid gland abnormalities, immunosuppression and carcinogenesis [10, 11]. With the persistence and toxic potential of pesticides influenced by various factors, including the chemical's resistance to degradation, its toxicity, and specific environmental conditions [12, 13].

Atrazine (ATZ), a widely used herbicide from the triazine group first developed by Geigy in 1958 [14] has become notable for its selective systemic action (photosystem-II (PSII)–inhibiting) on broadleaf weeds in various cultivated crops and uncultivated areas [15, 16]. The USEPA classifies atrazine as moderately toxic, and WHO categorizes it as an acute hazard under certain conditions [16, 17]. Atrazine's primary function disrupts photosynthesis and other enzymatic processes, though its widespread use has led to residual contamination in crops, soils, and various animal products, including milk and meat. Importantly, atrazine exposure affects endocrine functions in wildlife, raising concerns about ecosystem health and biodiversity [17].

Due to their biological resemblance to humans, guinea pigs (Cavia porcellus) are commonly used as experimental models and bio-indicators of environmental toxins, as they consume forage that may have been grown in pesticide-treated areas [18]. Despite being domesticated, guinea pigs' sensitivity to environmental pollutants allows them to serve as effective indicators of ecosystem contamination. Biochemical biomarkers in guinea pigs provide critical insights into cellular and physiological responses to xenobiotic exposure, offering early detection capabilities for assessing toxicological impacts [19].

Increasing research interest in natural, less toxic alternatives has brought attention to antioxidants from plants, which are abundant, cost-effective, and generally safer for biodiversity [20]. Notably, bitter leaf (Vernonia amygdalina) bitter leaf is recognized for its high antioxidant content, which may counteract the harmful effects of pesticide residues [21]. This study investigated effects of atrazine on the haematological, serum biochemical and oxidative stress profiles of guinea pigs (C. porcellus) and the ameliorative effect of aqueous bitter leaf extract, contributing to a broader understanding of pesticide impact on animal health and the potential role of plant-based antioxidants in mitigating toxicity of pesticides and or their residues in humans and animals.

MATERIALS AND METHODS

A total of 30 guinea pigs (C. porcellus), approximately three months old with a mean body weight of 402.00 ± 10.50g, were obtained from a certified breeder in Kaduna State, Nigeria. They were housed individually in standard cages (0.5m x 0.45m x 0.04m) and allowed to acclimatize for two weeks at the Animal House of the Department of Wildlife and Ecotourism, Forestry Research Institute of Nigeria, Ibadan. Prior to the

experiment, each guinea pig was weighed, and during the acclimatization and study periods, they were provided with water and forage ad libitum along with a supplement of Vital feed pelleted growers mash (Grand Cereals Limited). Animals were kept at an ambient temperature of 28°C with adequate air circulation and a 12-hour light/dark cycle. The experiment adhered to protocols approved by the University of Ibadan Animal Care and Use Research Ethics Committee (ACUREC), under the reference number UI ACUREC/18/0024. The study was carried out in eight weeks.

Fresh V. amygdalina leaves were collected from a cultivated farm in Ibadan, Oyo State, Nigeria, during the rainy season. A botanist authenticated the plant at the Forestry Herbarium Ibadan (FHI), where a voucher specimen (FHI. 11393) was deposited. Leaves were thoroughly rinsed with remove debris, air-dried for two weeks until brittle, then milled into powder and stored in airtight containers. The extract was prepared by infusing 2g of pulverized leaves in 180ml of hot water for 12 hours, then filtering through filter paper. A fresh infusion was prepared daily and administered to animals based on treatment dosage [22].

The 30 guinea pigs were randomly assigned to six groups (n=5 per group): one control group and four test groups receiving various treatments. The 30 guinea pigs were randomly assigned to six groups (n = 5 per group): one control group and five treatment groups. Animals in the treatment groups received atrazine at two dosages (low dose: 1,500 mg/kg BW and high dose: 3,000 mg/kg BW) and V. amygdalina extract at 1 ml/kg BW [23]. The treatment groups comprised Atrazine High (AH), Atrazine Low (AL), Atrazine High with aqueous Bitter Leaf Extract (AHB), Atrazine Low with aqueous Bitter Leaf Extract (ALB), aqueous Bitter Leaf Extract only (B), and Control (C). All groups received forage ad libitum. Atrazine was administered by incorporating the appropriate dose into the concentrate feed, whereas the aqueous V. amygdalina extract was administered separately by the oral route in drinking water. The two treatments were not mixed prior to administration (Table 1).

Blood samples were collected weekly via capillary tubes from the retro-orbital sinus. Samples were divided: one set in heparinized tubes for hematological analysis, including packed cell volume (PCV), hemoglobin concentration (Hb), red blood cell (RBC) count, and white blood cell (WBC) count, following standard methods (Dacie and Lewis, 1991); the other set in plain tubes was centrifuged at 5,000 rpm for 30 minutes to obtain obtain serum for biochemical assays. After final blood collection, three randomly selected guinea pigs per group were euthanized in a chloroform chamber, followed immediately by necropsy and tissue collection. After the final blood collection, three randomly selected guinea pigs per group were euthanized in a chloroform chamber and subjected to necropsy. Approximately 0.5 g of liver tissue was excised immediately, rinsed in ice-cold normal saline to remove blood, blotted dry, placed in labelled sample tubes, kept on ice, and subsequently homogenized for oxidative stress assays. Additional organs (lung, kidney, liver, heart, spleen, testis, adrenal, intestine, and brain) were fixed in 10% neutral buffered formalin for histopathological examination [24].

For histopathological evaluation, tissue samples from the lung, kidney, liver, heart, spleen, testis, adrenal, intestine and brain were processed, sectioned to 2 µm, and stained with hematoxylin and eosin. Tissue slides were examined and photographed under an Olympus CX21 light microscope by a veterinary pathologist. Additionally, 0.5 g of liver tissue from each animal was immediately preserved on ice post-extraction to prevent biomolecule degradation, enabling accurate oxidative stress measurement. Appropriate chemicals were utilized of scientific ratings and procedures adopted. Thereafter, readings were taken with spectrophotometer at specific wavelengths and documentation done. Activities of superoxide dismutase were assessed by evaluating auto-oxidation inhibition of epinephrine as outlined in [25] by Misra and Fridovich (1972). In accordance with the techniques outlined by Aebi (1983) and Rotruck et al. (1973), the operations of CAT, SOD and GPx were ascertained.

Atrazine (80% WP) used in this study, marketed as TANZINE, was procured from a licensed distributor (NAFDAC registration A5-1421). The commercial formulation was manufactured by Zhyiang Zhongsham Chemical Industry, China. All chemicals were obtained under licensed trade names.

Statistical Analysis

Hematological and biochemical data were analyzed with descriptive statistics and frequency counts using SPSS IBM version 20.0. Body weights were recorded weekly, and treatment doses were calculated based on body weight. Analysis of variance (ANOVA) was performed to test for significant differences at a 5% probability level in a completely randomised design.

Results

In the groups exposed to atrazine, there was a significant anaemia ($p<0.05$). Treatment with aqueous bitter leaf extract (V. amygdalina) mitigated the toxic effects of atrazine, normalizing packed cell volume

(PCV) in affected groups. Additionally, aqueous bitter leaf extract treatment increased the PCV in the control group ($p<0.05$). Although haemoglobin (Hb) concentration and red blood cell (RBC) counts in atrazine-exposed groups were moderately reduced, these values improved with aqueous bitter leaf extract intervention. White blood cell (WBC) counts, platelet counts and leukocyte differentials showed no significant variation between the atrazine-exposed and control groups ($p>0.05$). Aqueous bitter leaf extract intervention did, however, slightly decrease platelet and WBC counts by reducing neutrophil levels while modestly increasing lymphocyte counts. Also recorded was an increase in the neutrophil-lymphocyte ratio in the control group when compared to other groups (Table 2). The morphology of red blood cells (RBCs) remained normocytic and normochromic across all groups, showing no significant morphological deviations between atrazine-treated and control groups (Table 3).

Atrazine exposure at higher doses slightly increased total protein levels thereby causing dehydration, an effect reversed by aqueous bitter leaf extract treatment. Albumin levels followed a similar pattern. Serum enzyme levels, however, did not differ significantly across groups. Atrazine intoxication elevated serum creatinine and blood urea nitrogen (BUN) levels, as presented in Table 4. Total bilirubin levels were significantly increased ($p<0.05$) in the atrazine-exposed groups, indicating hyperbilirubinemia, which was alleviated in groups receiving aqueous bitter leaf extract intervention. Hypercholesterolemia was observed prominently in the atrazine-intoxicated groups ($p<0.05$), which aqueous bitter leaf extract treatment significantly reduced ($p<0.05$) in both high and low dose groups. Aqueous bitter leaf extract significantly reduced serum triglyceride concentrations in the low-dose atrazine group. HDL concentrations did not differ significantly between atrazine-treated and atrazine plus bitter leaf-treated groups, although the bitter leaf-only group exhibited a lower HDL concentration than most other groups. No significant differences were observed in LDL concentrations among the treatment groups (Table 5).

Atrazine exposure altered the oxidant–antioxidant status of the experimental animals. Hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), both indicators of oxidative stress, were generally higher in the atrazine-treated groups than in the control group. Reduced glutathione (GSH), an endogenous antioxidant, showed treatment-related variations across the experimental groups. Nitric oxide (NO) concentrations remained unchanged among the groups. Activities of the antioxidant enzymes glutathione-S-transferase (GST), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were significantly reduced ($p < 0.05$) in atrazine-treated animals compared with the control group (Table 6).

Hepatocellular atrophy and vacuolar degeneration of moderate to severe intensity were observed in the atrazine-treated animals, with severity varying by dose; no lesions were present in the aqueous bitter leaf extract and control groups (Table 7). Broncho-interstitial pneumonia was prevalent in the atrazine-exposed animals, though control and aqueous bitter leaf extract treated animals exhibited no such lesions. Other tissues, including spleen and myocardium, displayed notable histopathological effects: moderate lymphoid depletion in the spleen, necrotizing myocarditis in the heart, and mucous hyperplasia in atrazine-treated animals. No significant lesions were detected in other organs examined across treatment groups.

Discussion

This study investigates the hematological, biochemical, and histopathological impacts of atrazine exposure in guinea pigs (C. porcellus) and assesses the potential ameliorative effects of aqueous extract of bitter leaf (V.amygdalina).

The observed reduction in hematological indices, such as the PCV in atrazine-exposed groups, aligns with findings by [26] which highlight reduced PCV as indicative of an anemic state. The slight decrease in hemoglobin (Hb) and RBC counts further supports mild anemia induced by atrazine. This observation is consistent with [8] who also reported significant reductions in PCV, Hb, and RBC in guinea pigs exposed to cypermethrin, which were subsequently mitigated by aqueous extract of bitter leaf. [27] also demonstrated that V. amygdalina effectively counters chemical toxicity, reinforcing the extract's protective role against atrazine toxicity. Interestingly, while previous studies observed an increase in white blood cell counts in animals treated with bitter leaf diets [28] the current study noted reduced WBC and platelet counts due to lower neutrophil levels and a slight increase in lymphocyte counts, which may indicate an antibody response facilitated by aqueous bitter leaf extract, as supported by [29] in relation to its immunostimulant properties. Notably, while bitter leaf is reported to reduce prothrombin time and support hemostasis [30] this study found it resulted in thrombocytopenia, indicating its diverse effects on hematological parameters.

In biochemical assessments, atrazine exposure resulted

in hyperproteinemia, hyperalbuminemia, hyperbilirubinemia, and hypercholesterolemia compared to controls. These findings partly contradict those of [31], who reported mild decreases in similar biochemical parameters in toads. In contrast, the present study found no consistent alterations in AST, ALT, and ALP activities following atrazine exposure. Bitter leaf intervention significantly reduced triglycerides in atrazine-exposed groups ($p<0.05$), reinforcing its role in cholesterol regulation. This aligns with the therapeutic benefits of V. amygdalina in cholesterol control, potentially lowering risks of heart disease and stroke, as indicated by [30]. Previous research on hyperlipidemia reduction through bitter leaf extract also supports this finding [32], highlighting its benefits for cardiovascular health.

The antioxidative properties of V. amygdalina played a key role in countering atrazine-induced oxidative stress. Atrazine exposure was associated with elevated oxidative stress markers, particularly H_2O_2 and malondialdehyde (MDA), together with alterations in the antioxidant defence system, a pattern comparable to that reported by [32]. Histopathological findings revealed moderate to severe hepatocellular atrophy and vacuolar degeneration in atrazine-exposed animals, diverging from Omonona and Jarikre (2015), who found minimal pulmonary congestion in carbendazim-exposed rats. The ameliorative effect observed with aqueous bitter leaf extract in this study, however, supports [33], who highlighted the antioxidative potential of bitter leaf extract in reducing liver and kidney damage.

The anemia observed in the atrazine-exposed groups may be attributed to its disruption of hematopoiesis, which is consistent with the characterization of organochlorine pesticides as endocrine disruptors and carcinogens [34]. Increases in liver enzymes, hyperbilirubinemia, and hypercholesterolemia suggest hepatic injury, corroborating histological evidence. Additionally, elevated prooxidants such as reduced glutathione (GSH), hydrogen peroxide (H_2O_2), and malondialdehyde reflect oxidative stress, which, as reported by [5] is a hallmark of pesticide toxicity affecting physiological and reproductive health. This aligns with findings from [8] on increased malondialdehyde and reduced antioxidant activity in carbendazim-exposed animals, mitigated by antioxidants like vitamin E.

Conclusion

This study underscores the adverse hematological, biochemical, and histopathological effects of atrazine exposure in guinea pigs, evidenced by anemia, hyperbilirubinemia, hypercholesterolemia, and oxidative stress. The findings reveal that atrazine-induced oxidative stress manifests as elevated prooxidants and depleted antioxidant enzyme levels, contributing to cellular damage, especially in hepatic tissues. Importantly, aqueous extract of bitter leaf (Vernonia amygdalina) demonstrated a protective and restorative capacity, mitigating the toxic effects of atrazine with resultant amelioration of anemia, reduced serum cholesterol and bilirubin levels, and restored antioxidant enzyme activity. Histological assessments further confirmed that bitter leaf intervention could lessen the degree of hepatic and cellular damage, thereby supporting tissue recovery. These findings highlight bitter leaf's potential as a natural therapeutic agent in counteracting the toxic impacts of pesticide exposure, advocating for its consideration in integrative health strategies for managing environmental toxin exposure.

REFERENCES

1) Ogamba, E.N., Inyang, I.R. and Okechukwu, O.C. 2013. Effect of Kartodim 315ec (Dimethoate) and Lambda-(Cyhalothrin) On electrolytes of Clarias gariepinus. IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 5(2): 1 – 4.
2) Bhatt, P., Joshi, T., Bhatt, K., Zhang, W., Huang, Y. and Chen, S. 2021. Binding interaction of glyphosate with glyphosate oxidoreductase and C-P lyase: Molecular docking and molecular dynamics simulation studies. Journal of Hazardous Materials; 409:124927. https://doi.org/10.1016/j.jhazmat.2020.124927
3) Zhang, W., Pang, S., Lin, Z., Mishra, S., Bhatt, P. and Chen, S. 2021. Biotransformation of perfluoroalkyl acid precursors from various environmental systems: Advances and perspectives. Environmental Pollution 272:115908. https://doi.org/10.1016/j.envpol.2020.115908
4) Pathak, V. M., Verma, V.K., Rawat, B. S., Kaur, B., Babu, N., Sharma, A., Dewali, S., Yadav, M., Kumari, R., Singh, S., Mohapatra, A., Pandey, V., Rana, N. and Cunill, J. M., 2022. Current status of pesticide effects on environment, human health and it's eco-friendly management as bioremediation: A comprehensive review. Frontier in Microbiology https://doi.org/10.3389/fmicb.2022.962619
5) Omonona, A. O., Jarikre, T. A. and Adetuga, A. T. 2015. Clinico-pathological effects of single oral dose of cypermethrin in guinea pigs. Sokoto Journal of Veterinary Sciences, 13 (1): 1 – 8.

6) Chung, S. W. and Chen, B. L. 2011. Determination of organochlorine pesticide residues in fatty foods: a critical review on the analytical methods and their testing capabilities. Journal of Chromatography A;1218(33):5555-67. https://doi.org/10.1016/j.chroma.2011.06.066
7) Burger, J., Mol, F. and Gerowitt, B. 2008. The 'necessary extent' of pesticide use -Thoughts about a key term in German pesticide policy. Crop Protection, 27: 343 – 351.
8) Omonona, A. O. and Jarikre, T. A. 2015. Comparative toxicity of cypermethrin following oral and dermal routes in guinea pigs and the amelioration effect of ascorbic acid. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 6(1): 1552 – 1556.
9) Kalyabina, V. P., Esimbekova, E. N., Kopylova, K. V. and Kratasyuk, V. A. 2021. Pesticides: formulants, distribution pathways and effects on human health–a review. Toxicology Report 8, 1179–1192. https://doi.org/10.1016/j.toxrep.2021.06.004
10) Kori, R. K., Singh, M. K., Jain, A. K. and Yadav, R. S. 2018. Neurochemical and behavioral dysfunctions in pesticide exposed farm workers: a clinical outcome. Indian Journal of Clinical Biochemistry 33(4):372–381 https://doi.org/10.1007/s12291-018-0791-5
11) Brandt, A., Hohnheiser, B., Sgolastra, F., Bosch, J., Meixner, M. D. and Buchler, R. 2020. Immunosuppression response to the neonicotinoid insecticide thiacloprid in females and males of the red mason bee Osmia bicornis L.. Scientific Reports 10:1-10. https://doi.org/10.1038/s41598-020-61445-w
12) Akegbejo, S. Y. 1996. Introduction to Wildlife Management in Nigeria (First edition). Giad Educational Publisher Abeokuta, Pp. 55 – 59.
13) Omonona, A. O. and Kayode, I. B. 2011.Wildlife Health. In: Ecotourism Implications on Human and Wildlife Health. First edition. Ibadan, Ibadan University Press. Pp 37 – 41.
14) IPCS, 1990. International Programme on Chemical Safety. Atrazine Health and Safety Guide No.47, WHO Geneva. P. 46.
15) USEPA, 1994. United States Environmental Protection Agency. Atrazine, simazine and cyanosine: notice of initiation of special review, Federal Registry, 59: 60412 – 60443.
16) IPCS, 1999. International Programme on Chemical Safety. The WHO recommended classification of pesticides by hazard and guidelines to classification 1998-1999, WHO/IPCS/98.21.
17) USEPA, 2003. United States Environmental Protection Agency. Atrazine Interim Reregistration Eligibility Decision. http://www.epa.gov/oppsrrd1/REDs/atrazine_ired.pdf.
18) Lakra, W. S. and Nagpure, N. S. 2009. Genotoxicological studies in fishes: A review. Indian Journal of Animal Science, 79: 93 – 98.
19) Monserrat, J. M., Martinez, P. E., Geracitano, L. A., Amado, L. L., Martins, C. M. G., Pinho, G. L. L., Chaves, I. S., Ferreira-Cravo, M., Ventura-Lima, J. and Bianchini, A. 2007. Pollution biomarkers in estuarine animals: critical review and new perspectives. Comparative Biochemistry and Physiology, 146C: 221 – 234. https://doi.org/10.1016/j.cbpc.2006.08.012
20) Oyewole, I. O., Magaji, Z. and Awoyinka, O. A. 2007. Biochemical and toxicological studies of aqueous extract of Tithonia diversifolia (Hemsl) leaves in Wister albino rats. Journal of Medicinal Plant Research, 1(2): 030 – 033.
21) Favi, F., Cantrell, C. I., Mebrahtu, T. and Kraemer, M. E. 2008. Leaf peltate glandular trichomes of Vernonia galamensis ssp. Galamensis var ethiopica gilbert: Development, Ultrastructure, and chemical composition. International Journal of Plant Science, 169: 605 – 614.
22) Imaga, N. O. A. and Bamigbetan, D. O. 2013. In vivo biochemical assessment of aqueous extracts of Vernonia amygdalina (Bitter leaf). International Journal of Nutrition and Metabolism, 5 (2): 22 – 27.
23) Sajad, F. D., Hassan, M., Rasool, S. and Rajab, A. S. 2011. Detrimental effect of atrazine on testicular tissue and sperm quality: implication for oxidative stress and hormonal alterations. Iranian Journal of Toxicology 5(12), 426–435.
24) Akpavie, S. O. 2004. General veterinary pathology notes for undergraduates. (First edition). Stirling-Horden Publishers Nigeria Limited. Pp 9 – 10.
25) Oyagbemi, A. A., Omobowale, T. O., Akinrinde, A. S., Saba, A. B., Ogunpolu, B. S. and Daramola, O. 2015. Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. Environmental Toxicology, 30 (11): 1235 – 1243.
26) Christian, S. G., Elekima, I., Obisike, U. A. and Aleru, C. P. 2016. Effect of petroleum on haematological parameters and lead level in fuel attendants in Port Harcourt, Nigeria. International Journal of Scientific Research, 5 (3): 280 – 283.
27) Ikeh, C. K., Ikeh, P. E. and Ezike, C. A. 2014. Protective potential of aqueous leaf extract of Vernonia amygdalina in cyclophosphamide–induced myelotoxicity. International Organization

of Scientific Research Journal of Pharmacy, 4 (3):6 – 14.
28) Okoye, J. O., Ngokere, A. A., Chizoba, O. and Okeke, C. O. 2014. Biochemical, haematological and histological effects following Escravos crude oil ingestion by Chinchilla rabbits International Journal of Medicine and Medical Sciences, 6 (2): 63 – 68.
29) Osho, I. B., Akindahunsi, A., Igbasan, F. A. and Adekunle, D. J. 2014. Effect of orally administered bitter leaf (Vernonia amygdalina) extract on the growth performance and haematological parameters of broiler chicken. Journal of Veterinary Medicine and Animal Health. 6(10):251 – 256.
30) Oguwike, F. N., Offor, C. C., Onubeze, D. P. M. and Nwadioha, A. N. 2013. Evaluation of Activities of Bitterleaf (Vernonia amygdalina) Extract on Haemostatic and Biochemical Profile of Induced Male Diabetic Albino Rats. Journal of Dental and Medical Sciences (IOSR- JDMS), 11(2): 60 – 64.
31) Omonona, A. O and Emikpe, B. O. 2011. Clinicopathological Features Associated with Acute Toxicity of Lambda-cyhalothrin Pesticide in Adult Toads (Buffo Perreti). Advances in Environmental Biology, 5 (5): 808 – 813.
32) Oseni, K. and Babatunde, O. 2016. Vernonia amygdalina: An Underutilized Vegetable with Nutraceutical Potentials – A Review. Turkish Journal of Agriculture - Food Science and Technology, 4 (9): 763 – 768.
33) Minari, J. B. 2012. Hepatoprotective effect of methanolic extract of Vernonia amygdalina leaf. Journal Natural Products, 5: 188 – 192.
34) Lemaire, G., Terouanne, B., Mauvais, P., Michel, S. and Rahmani, R. 2004. Effect of organochlorine pesticides on human androgen receptor activation in vitro. Toxicology and Applied Pharmacology, 196: 235 – 246.

Table 1: Experimental layout and treatment combinations

Group	Pesticide (Atrazine)	Combinations
I	Low dosage	1,500 mg/kg BW
II	High dosage	3,000 mg/kg BW
III	Low dosage + Bitter leaf extract	I + 1 ml/kg bitter leaf extract
IV	High dosage + Bitter leaf extract	II + 1 ml/kg bitter leaf extract
V	Bitter leaf extract only	1 ml/kg bitter leaf extract
VI	Control	No treatment

Table 2: Haemogram Changes in the Experimental Animals Exposed to Different Levels of Pesticide and Aqueous Extract of Bitter Leaf

Group	Treatment					
	I	II	III	IV	V	VI
PCV	36.00 ± 2.16^{a}	35.50 ± 2.41^{ab}	40.00 ± 2.16^{bc}	37.01 ± 2.42^{bc}	42.75 ± 2.41^{c}	40.00 ± 2.41^{bc}
HB	12.16 ± 0.48^{a}	12.63 ± 0.54^{ab}	13.00 ± 0.48^{bc}	13.03 ± 0.71^{bc}	12.95 ± 0.54^{c}	12.25 ± 0.54^{ac}
RBC	6.79 ± 0.20^{a}	7.05 ± 0.22^{a}	7.60 ± 0.20^{a}	7.51 ± 0.20^{a}	6.42 ± 0.22^{a}	7.25 ± 0.22^{a}
WBC (x10^{3}µL)	5.59 ± 0.75^{a}	5.06 ± 0.84^{ab}	5.00 ± 0.75^{ab}	4.58 ± 0.42^{a}	4.03 ± 0.84^{b}	5.57 ± 0.84^{ab}
Platelet (x10^{5}µL)	1.55 ± 0.73^{a}	1.47 ± 0.82^{a}	2.50 ± 0.73^{b}	2.43 ± 0.80^{a}	0.76 ± 0.82^{a}	0.74 ± 0.82^{a}
Lymph	66.20 ± 2.08^{a}	69.00 ± 2.32^{ab}	68.80 ± 2.08^{a}	70.05 ± 2.12^{a}	73.75 ± 2.32^{b}	64.75 ± 2.32^{a}
Neutro	35.40 ± 2.16^{a}	33.50 ± 2.42^{ab}	34.20 ± 2.16^{a}	34.01 ± 2.17^{a}	27.25 ± 2.42^{b}	34.00 ± 2.42^{a}
Neutro:Lymph Ratio	0.42 ± 0.02	0.39 ± 0.02	0.42 ± 0.02	0.45 ± 0.06	0.42 ± 0.02	0.58 ± 0.06
Mono	1.40 ± 0.33^{abc}	1.50 ± 0.37^{ac}	1.60 ± 0.33^{abc}	1.58 ± 0.34^{ab}	0.50 ± 0.37^{b}	2.00 ± 0.37^{c}
Eosin	0.80 ± 0.38^{a}	0.75 ± 0.43^{a}	0.40 ± 0.38^{a}	0.68 ± 0.35^{a}	0.75 ± 0.43^{a}	0.75 ± 0.43^{a}

Cells in same row with the same superscript are not statistically significantly different

NOTE: Packed Cell Volume- PCV (%), Haemoglobin Concentration-Hb (g/dl), Red Blood Cell-RBC (*103/ µL), White Blood Cell-WBC (*103/ µL), Platelet Count-Plate (*105/ µL), Lymphocytes-Lym, Neutrophils-Neutro, Monocytes-Mono, Eosinophils-Esino.

I: Atrazine high; II: Atrazine low; III: Atrazine high with aqueous bitter leaf extract; IV: Atrazine low with aqueous bitter leaf extract; V: Aqueous Bitter leaf extract; VI: Control

Table 3: Red Cell Indices in the Experimental Animals Exposed to Different Levels of Pesticide and Aqueous Bitter Leaf Extract

Group	Treatment					
	I	II	III	IV	V	VI
MCV	61.51 ± 0.88^{a}	59.64 ± 0.99^{ab}	60.00 ± 0.88^{b}	59.51 ± 0.88^{ab}	58.03 ± 0.99^{b}	59.69 ± 0.99^{ab}
MCH	19.69 ± 0.52^{a}	19.78 ± 0.58^{a}	20.20 ± 0.52^{a}	19.30 ± 0.50^{a}	19.19 ± 0.58^{a}	19.38 ± 0.58^{a}
MCHC	32.54 ± 0.34^{ab}	32.37 ± 0.38^{a}	33.00 ± 0.34^{bc}	33.00 ± 0.32^{c}	32.86 ± 0.38^{bc}	33.02 ± 0.38^{abc}

Cells in same row with the same superscript are not statistically significantly different

NOTES: Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin-MCH (%), Mean Cell Haemoglobin Concentration-MCHC (pg)

I: Atrazine high; II: Atrazine low; III: Atrazine high with aqueous bitter leaf extract; IV: Atrazine low with aqueous bitter leaf extract; V: Aqueous bitter leaf extract; VI Control

Table 4: Serum Chemistry Changes in the Experimental Animals Exposed to Different Levels of Pesticides and Aqueous Bitter Leaf Extract

Group	Treatment					
	I	II	III	IV	V	VI
TP	7.28 ± 0.24^{a}	6.70 ± 0.26^{a}	6.60 ± 0.24^{a}	6.67 ± 0.24^{a}	6.48 ± 0.26^{a}	6.68 ± 0.26^{a}
ALB	3.16 ± 0.14^{a}	2.85 ± 0.16^{a}	2.80 ± 0.14^{a}	2.51 ± 0.16^{a}	2.63 ± 0.18^{a}	2.75 ± 0.16^{a}
GLOB	4.00 ± 0.16^{a}	4.08 ± 0.18^{a}	4.00 ± 0.16^{a}	4.00 ± 0.17^{a}	3.78 ± 0.18^{a}	3.90 ± 0.18^{a}
AST	35.60 ± 4.31^{ab}	33.00 ± 4.82^{ab}	33.60 ± 4.31^{ab}	31.02 ± 4.80^{ab}	34.00 ± 4.82^{a}	43.50 ± 4.82^{b}
ALT	35.00 ± 2.27^{ac}	37.00 ± 2.54^{ac}	39.60 ± 2.27^{abc}	39.01 ± 2.54^{b}	40.00 ± 2.54^{abc}	39.50 ± 2.54^{c}
ALP	65.40 ± 4.09^{a}	67.25 ± 4.57^{a}	68.20 ± 4.09^{a}	66.40 ± 4.09^{a}	66.50 ± 4.57^{a}	65.00 ± 4.57^{a}
BUN	15.36 ± 1.60^{a}	13.13 ± 1.78^{a}	13.60 ± 1.60^{a}	12.31 ± 1.78^{a}	12.23 ± 1.78^{a}	12.60 ± 1.78^{a}
CREAT	0.76 ± 0.04^{a}	0.68 ± 0.05^{a}	1.00 ± 0.04^{a}	0.65 ± 0.05^{a}	0.65 ± 0.05^{a}	0.75± 0.05^{a}
AG Ratio	0.66 ± 0.03^{a}	0.68 ± 0.03^{a}	1.00 ± 0.03^{a}	0.07 ± 0.03^{a}	0.70 ± 0.03^{a}	0.70 ± 0.03^{a}

Cells in same row with the same superscript are not statistically significantly different

NOTES: Mean Cell Volume-MCV (fl), Mean Cell Haemoglobin-MCH (%), Mean Cell Haemoglobin Concentration-MCHC (pg)

I: Atrazine high; II: Atrazine low; III: Atrazine high with aqueous bitter leaf extract; IV: Atrazine low with aqueous bitter leaf extract; V: Aqueous bitter leaf extract; VI: Control

Table 5: Serum Lipid Changes in the Experimental Animals Exposed to Different Levels of Pesticides and Aqueous Bitter Leaf Extract

Group	**Treatment**					
	I	II	III	IV	V	VI
Total Bilirubin	0.60 ± 0.42[a]	5.90 ± 0.47[a]	5.60 ± 0.42[a]	5.22 ± 0.42[a]	0.75 ± 0.47[a]	0.50 ± 0.47[a]
Cholesterol	42.60 ± 5.33[a]	43.25 ± 5.96[a]	47.20 ± 5.33[a]	42.35 ± 5.96[a]	2.43 ± 5.96[a]	2.20 ± 5.96[a]
Triglycerol	50.40 ± 3.20[a]	39.50 ± 3.58[a]	46.00 ± 3.20[a]	37.67 ± 3.58[a]	38.00 ± 3.58[a]	45.25 ± 3.58[a]
High Density Lipoprotein (HDL)	26.80 ± 2.96[a]	26.75 ± 3.31[a]	29.00 ± 2.96[a]	26.55 ± 3.30[ab]	20.00 ± 3.31[b]	26.75 ± 3.3[a]
Low Density Lipoprotein (LDL)	3.80 ± 2.03[a]	4.10 ± 2.27[a]	3.20 ± 2.03[a]	3.98 ± 2.03[a]	21.00 ± 2.27[a]	27.75 ± 2.27[a]

Cells in same row with the same superscript are not statistically significantly different

Total bilirubin (mg/l) and Cholesterol (mg/l)

I: Atrazine high; II: Atrazine low; III: Atrazine high with aqueous bitter leaf extract; IV: Atrazine low with aqueous bitter leaf extract; V: Aqueous bitter leaf extract; VI: Control

Table 6: Antioxidant and Pro-oxidant Parameters in the Experimental Animals Exposed to Different Levels of Pesticides and Bitter Leaf

GROUP	**TOTAL PROTEIN**	**GSH**	**NO**	**H_2O_2**	**MDA**	**GST**	**SOD**	**GPx**
I	2.71±1.23	74.64±1.19	125.14±0.00	67.31±12.90	3.68±0.26	1.68±0.11	7.63±0.18	246.61±7.17
II	2.18±0.41	66.27±0.82	125.14±0.00	89.73±29.10	0.80±0.12	0.08±0.04	3.35±0.76	107.47±23.60
III	1.54±0.15	76.62±1.74	125.14±0.00	62.73±8.01	1.25±0.22	1.21±0.39	4.45±0.42	140.11±12.50
IV	1.22±0.12	66.62±1.47	125.14±0.00	73.37±4.01	0.25±0.22	1.21±0.39	4.58±0.18	145.09±15.89
V	1.16±0.13	70.17±1.45	125.14±0.00	48.14±5.21	1.18±0.32	0.23±0.07	6.28±0.62	189.20±18.80
VI	1.29±0.13	69.60±1.80	125.14±0.00	47.31±2.22	1.26±0.16	0.54±0.28	5.36±0.09	167.75±1.39

I: Atrazine high
II: Atrazine low
III: Atrazine high with aqueous extract of bitter leaf
IV: Atrazine low with aqueous extract of bitter leaf

V: Aqueous extract of bitter leaf

VI: Control

TP- Total Protein (mg/ml)
GPx- Glutathione peroxidase (units mg^{-1} protein);
GSH- Reduced glutathione ($mgml^{-1}$)
GST-Glutathione transferase (unit mg^{-1} protein)
SOD- Superoxide dismutase (unit mg^{-1} protein)
(MDA)- Malondialdehyde (µmol/ml)
Hydrogen peroxide generation (µmol mg^{-1} protein)
NO – Nitric oxide (µmol mg^{-1} protein)

Liver	Kidney	Lung	Spleen	Heart	Brain	Intestine	Adrenal	Testis
Diffuse vacuolar degeneration of hepatocytes	No observable lesion	Moderate interstitial pneumonia	No observable lesion	Myofibre degeneration	No observable lesion	Mucous hyperplasia	No observable lesion	No observable lesion
Moderate diffuse hepatocellular atrophy	No observable lesion	Mild interstitial pneumonia	No observable lesion	Necrotizing myocarditiss	No observable lesion	Goblet hyperplasia	No observable lesion	No observable lesion
No observable lesion	No observable lesion	No observable lesion	Lymphoid hyperplasia	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion
No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion
No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion
No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion	No observable lesion