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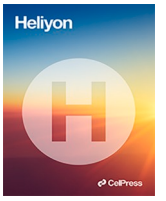

Immune boosting and ameliorative properties of aqueous extract of *Vernonia amygdalina* Delile against MSG-induced genotoxicity: An *in silico* and *in vivo* approach

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ABSTRACT

Vernonia amygdalina (VA) is popularly consumed as food and as medicine due to its nutritional and bioactive constituents. This study assessed the *anti*-genotoxic effect of aqueous leaf extract of VA against monosodium (MSG) –induced genotoxicity. Crude extraction and phytochemical analysis were done using standard methods. *In silico* studies was done using compounds in the extract against Bcl-2, NF-kB 50, DNA polymerase lambda, DNA ligase, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). Twelve rats were divided into three groups with four rats in each group. Group I was fed on food and water, group II received MSG (4 g/kg) per body weight (pbw) intraperitoneally, group III received MSG (4 g/kg) pbw intraperitoneally followed by oral dose of VA leaf extract (250 mg/kg) per body weight. The number of the micronucleated red blood cells and white blood cells were determined from blood smears microscopically. Results showed that aqueous extract of VA contained in mg/100 g alkaloids (7.04 ± 0.16), saponins (3.91 ± 0.13), flavonoid (1.64 ± 0.16), phenol (3.40 ± 0.12) and tannins (0.07 ± 0.32). In silico studies revealed high binding interaction ($\Delta G > -8.6$) of vernoniosides D and E with all the tested proteins. There was a reduction in the number of micronucleated cells, neutrophils and eosinophils of the treated group compared to the MSG group, while there was an increase in the lymphocyte count. The *anti*-genotoxic effects of VA leaf extract might be attributed to the synergistic interaction of the various bioactive components in the extract. VA could be a potential plant for the prevention of cancer and other diseases that attenuate the immune system.

1. Introduction

Reactive oxygen species (ROS) which are also known as free radicals are formed through the metabolism of various endogenous molecules and other exogenous agents that enter into the body system. More than often, excess production of these ROS creates an overwhelming imbalance which cannot easily be adjusted by the intracellular antioxidant defense system leading to oxidative stress.

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These radicals are capable of forming adducts with various molecules in the body such as protein, lipid and DNA resulting in disruption of the normal cellular functions of these biomolecules [1]. Moreover, reactive oxygen species also impede the proper functioning of repair system by preventing DNA from binding to specific transcription factors. ROS alters the capacity of some transcription factors to bind DNA which hinders competent repair processes in the cell [2]. Eventually, this might cause deleterious effects on the DNA resulting in genotoxicity. Damage to DNA, the reservoir of the genetic information in a living organism as a result of exposure to genotoxic agents can lead to deleterious effects in the body system. The immune system can also be affected by genotoxic agents and this might result in either activation or suppression of the immune system [3]. Different agents have been implicated in genotoxicity. Genotoxic agents are capable of inducing deleterious changes in either DNA or RNA resulting in compromised integrity of the cell [4, 5]. An example of genotoxic agent is the common flavor enhancer used in food -the sodium salt of glutamic acid, popularly called monosodium glutamate (MSG) [6–8].

Damage to the genetic material has been connected to the origin of some diseases such as cancer, heart diseases and neurological impairments [9]. There is therefore a dire need for bioactive principles from natural sources which are cheaper, safer and non-toxic that can be used in combating genotoxicity-induced diseases.

Vernonia amygdalina is one of the vegetables fit for human consumption commonly consumed in Nigeria and other areas of African sub regions [10]. It is a member of the Asteraceace family and grows majorly in different ecological zones in the tropics of Africa [11]. The high concentration of bitter principles in it justifies the reason why it is called bitter leaf [12]. Due to its medicinal principles, it has been reported to demonstrate medicinal effects in the treatment of various ailments such as diabetes [13]; hypertension [14]; inflammation [15]; liver toxicity [16] and oxidative stress [17]. There is still paucity of information on the *anti*-genotoxic potential of *V. amygdalina* and the likely mechanisms involved, hence this study was conducted to give more insight on the *anti*-genotoxic potential of *V. amygdalina* and the probable mechanisms involved by employing molecular docking tools to determine the interactions of the main chemical compounds in *V. amygdalina* with some anti-apoptotic proteins and DNA repair proteins. Both anti-apoptotic proteins and DNA repair enzymes play important roles in ameliorating the genotoxic effects of genotoxic substances. Molecular docking is an *in silico* approach that provides more insight on the mechanism of action of medicinal plants by studying the interaction of the chemical compounds in the plants with specific target proteins otherwise known as receptors in order to predict and substantiate the medicinal effects of the plants [18].

Different tests are commonly employed as biomarkers of genetic changes induced by genotoxic agents. A common test is the micronucleus test which is used for detecting changes in the gene. Micronuclei are acentric fragments of chromosome or intact chromosomes that do not get included within the nucleus in the final stage of mitosis [19]. The erythrocyte micronucleus assay is commonly employed as a marker of genotoxicty because it is easier and when mice are used, a better result is obtained as mice do not clear micronucleated erythrocytes from the circulation completely [20].

This study was targeted at evaluating the immune-boosting and ameliorative properties of aqueous extract of *Vernonia amygdalina* against MSG-induced genotoxicity in rats.

2. Methodology

2.1. Plant identification and preparation

A sample of the plant specimen was authenticated as *Vernonia amygdalina* Delile at the Department of Botany, University of Lagos, Akoka, Nigeria where it was assigned the voucher number 9989. The plant was then prepared as described by Ref. [21] with slight adjustment. Fresh, matured *Vernonia amygdalina* leaves were harvested from a vegetable garden at Ipetumodu, Osun State, Nigeria. The leaves were sun-dried for four days. The dried leaves were blended into powder. One liter of distilled water was added to 100 g of the powdered bitter leaves in a volumetric flask. The mix was then allowed to stand for 72 h, followed by filtration using a filter paper and funnel into a conical flask. The filtrate obtained was then concentrated in a rotary evaporator at a temperature of 70 °C and kept till when needed.

*2.2. **Phytochemical analysis**: the following phytochemical analyses were performed on the aqueous leaf extract of V. amygdalina*

2.2.1. Determination of flavonoids

This was done according to Ref. [22]. One hundred ml of 80 % aqueous methanol was used to extract 10 g of VA leaf successively at room temperature. This was followed by filtration using filter paper. The filtrate was then evaporated in a water bath and the final weight determined.

% Flavonoids concentration = (Weight of Final Filtrate)/(Weight of Sample) X 100

2.2.2. Determination of alkaloid content

Two hundred ml of 20 % acetic acid in ethanol was added to about 5 g of *V. amygdalina* leaf powder and left for 4 h. Afterwards, the extract was filtered and then concentrated to 25 % of its original volume in a water bath. To the extract was then added concentrated ammonium hydroxide dropwise until there was total precipitation. The whole solution was left to settle and ammonium hydroxide was used to wash the precipitate collected followed by filtration. The precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The dry weight of the residue obtained which is the alkaloid was then determined [22].

2.2.3. Saponin content determination

This was done following the method of [22]. One hundred ml of 20 % aqueous ethanol was added to 20 g of *V. amygdalina* leaf powder inside a conical flask. It was then allowed to boil over a hot water bath and stirred continuously for 4 h at about 550 °C. This was followed by filtration and the re-extraction of the residue with 20 % ethanol (200 ml) until there was a reduction in the volume to 40 ml upon boiling in a water bath at about 900 °C. 20 ml of diethyl ether was added to the concentrate in a 250 ml separating funnel and shaken strongly followed by the recovery of the aqueous layer. The whole experiment was repeated followed by the addition of N-butanol. Ten ml of 5 % aqueous sodium chloride was used to wash all the N-butanol extracts collected and then boiled in a water bath to allow for evaporation of the solvent. This was followed by drying in the oven until a constant weight was achieved. The saponins content was determined using the formular below:

% Saponins concentration = (Weight of Final Filtrate)/(Weight of Sample) X 100

2.2.4. Determination of phenol

The phenolic content was extracted by boiling the sample devoid of fat with 50 ml ether for 15 min. Ten ml distilled water was then added to 5 ml of the extract in a 50 ml flask. This was followed by the addition of 2 ml ammonium hydroxide solution and 5 ml of concentrated amyl alcohol. After being made up to mark, the sample was then allowed to settle for the reaction to occur. Development of color was measured after 30 min in a spectrophotometer as 505 nm [23].

2.2.5. Tannin content determination

This was carried out as spelt out by Ref. [24]. To 500 mg of *V. amygdalina* leaf powder in a 50 ml plastic bottle was added 50 ml of distilled water. The mixture was shaken for 1 h in a mechanical shaker. This was then made up to the mark after filtration into a 50 ml volumetric flask. To 5 ml of the filtrate was added 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M Potassium ferrocyanide which were mixed together. After 10 min, the absorbance was read at 120 nm.

2.3. In silico studies

The main active constituents in *V. amygdalina* leaf as obtained from previous reports by Refs. [25–27] were used for the molecular docking studies. The structural configurations of these compounds were obtained from PubChem (http://pubchem.ncbi.nlm.nih.gov) database in SDF format after which PYMOL was used to change them into pdb files. Afterwards, the pdbqt files of the ligands were produced using MGL Tools 1.5.4, as the number of rotatable bonds were set to maximum and then subjected to molecular docking studies. The structures of MSG and ascorbic acid were also docked as the negative and positive control respectively. The target proteins include anti-apoptotic proteins- Bcl-2 and NF-kB 50, DNA repair proteins- DNA polymerase lambda and DNA ligase and the antioxidant enzymes-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) (Table 1). The PDB forms of the 3D structures of the target proteins were downloaded from RCSB Protein Data Bank. The protein structures were set as proper targets, using the Autodock Tools (version 1.5.6, The Scripps Research Institute, La Jolla, CA, USA) [28]. Molecules of H_2O, metal atoms, co-crystallized ligands and unwanted protein chains were removed from the protein using Pymol. Polar hydrogen atoms and Gesteiger charges were then added. Afterwards, the docking grid maps were generated using the Autodock MGL Tools 1.5.4. The grid box, which confines the

Table 1
Molecular docking parameters and protein targets.

	Protein	PDB ID	Grid Box Center Coordinates	Grid box size
Anti-apoptotic proteins				
	BCl-2	6O0K	center_x = −3.452	size_x = 50
			center_y = 1.517	size_y = 40
			center_z = −11.441	size_z = 42
	NF-kB 50	1BFS	center_x = 46.999	size_x = 46
			center_y = 22.668	size_y = 44
			center_z = 5.596	size_z = 40
DNA	Human Polymerase lambda	5CB1	center_x = 206.32	size_x = 40
Repair enzymes			center_y = 176.529	size_y = 40
			center_z = 153.3	size_z = 40
	DNA ligase	3PC7	center_x = −7.749	size_x = 40
			center_y = 7.854	size_y = 40
			center_z = 0.664	size_z = 40
Antioxidant enzymes	Superoxide dismutase (SOD)	ICB4	center_x = 20.566	size_x = 30
			center_y = 67.616	size_y = 42
			center_z = 13.888	size_z = 28
	Catalase (CAT)	2CAG	center_x = 62.071	size_x = 40
			center_y = 17.869	size_y = 36
			center_z = 15.834	size_z = 44
	Glutathione Peroxidase (GPX)	2P31	center_x = −8.168	size_x = 20
			center_y = −1.793	size_y = 20
			center_z = −23.796	size_z = 38

target space, was specified in terms of coordinates and size as shown in Table 1, taking into consideration the amino acids at the active site of the enzyme. The target protein was saved as the required file format (pdbqt). The dockings were done using AutoDock Vina 1.1.2 [28], a modern day docking program produced by the Molecular Graphics Laboratory. Pymol and discovery studio were employed for the analyses of docking outputs. The binding energy and intermolecular interactions were compared and analyzed using BIOVIA discovery studio visualizer (version 19). Results obtained from the docking analysis were represented as (ΔG, kcal/mol), which is the value of the free energy generated from the binding of the respective ligands to the respective proteins.

2.4. Experimental animals

A total of twelve Wistar strain albino rats were used for this experiment. The rats were eight weeks old at the time they were kept in well ventilated plastic cages and were made to adapt to the environment for a period of two weeks under the conditions of temperature (26 ± 2 °C), humidity (60 ± 5 %) and photoperiodicity of 12 h light/12 dark cycle. They had unrestricted access to standard pellet and water. The experimental methods were carried out in conformity to the guidelines of the Institutional Animal Ethics Committee (IAEC) with the approval number U/16/BC/0474.

2.4.1. Experimental design

At the end of the acclimatization period, the rats were randomly divided into three groups with four rats in each group as follows: .

Group I (Control group): Were fed only food and water.

Group II (MSG only group): The rats were administered monosodium glutamate (MSG) intraperitoneally at a concentration of 4 g/kg per body weight of the rats.

Group III (MSG + *V. amygdalina* extract): The rats were first given 4 g/kg per body weights of MSG and after 24 h were then treated with aqueous extract of *Vernonia amygdalina* (250 mg/kg per body weight).

2.5. Anti-genotoxicity test and white blood cell count determination

At the end of the experiment, the animals were anaesthetized lightly using ether and then sacrificed by cervical dislocation. Specimens of blood were obtained by direct cardiac perforation and analyzed to evaluate the *anti*-genotoxicity potential of *V. amygalina* by determining the number of micronucleated red blood cells using a 100× oil immersion objective lens of a compound light microscope, which was also used to evaluate the number of the lymphocytes, neutrophils and eosinophils.

2.6. Statistical analysis

The results obtained were expressed as Mean ± SD of triplicate analysis. One way analysis of variance (ANOVA) was then used to compare the mean differences between and within the groups and a $p < 0.05$ was considered significant.

3. Result

3.1. Phytochemical analysis

The result of the phytochemical analysis of the leaves of *Vernonia amygdalina* is shown in Table 2. As seen in the table, the leaf is richest in alkaloids followed by saponins. However, the leaf is very low in tannins.

3.2. In silico studies

The results of the *in silico* molecular docking of the target proteins with the compounds in *V. amygdalina* leaf is depicted in Table 3 as a heat map table. The heat map table was constructed using a blue-yellow color format in which the lowest negative energy values are written in blue while the highest negative energy values are highlighted in yellow. This is to permit quick recognition of compounds that interact more strongly with the target proteins as they have higher negative values than other compounds in the extract.

The structures of the lead compounds in *V. amygdalina* leaf with high affinities for anti-apoptotic proteins, DNA repair enzymes and antioxidant enzymes are shown in Fig. 1(a–d).

Table 2
Phytochemical constituents of *Vernonia amygdalina* leaf.

Phytochemicals	*Vernonia amygdalina* (mg/100 g)
Flavonoids	1.64 ± 0.16
Alkaloids	7.04 ± 0.16
Saponins	3.91 ± 0.13
Phenol	3.40 ± 0.12
Tannins	0.07 ± 0.32

Values are Mean ± SD of three determinations.

Table 3
Heat map of recorded docking scores (binding free energy—kcal/mol) of the chemicals contained in *V. amygdalina* leaf.

S/N	Compounds in *V. amygdalina* leaf	Bcl-2 6O0K	NFkB-50 1BFS	Polymerase 5CB1	DNA Ligase 3PC7	SOD 1CB4	CAT 2CAG	GPX 2P31
1	Squalene	−6.2	−4.4	−4.6	−6.4	−6.2	−8.9	−7.9
2	Beta -sitosterol	−7.8	−6.1	−6.3	−8.2	−8.3	−8.9	−5.9
3	Methyl-2-*O*-benzyl-D-arabinofuranoside	−6.1	−5.1	−5.7	−6.6	−6.5	−6.9	−5.3
4	Stearic acid	−6.0	−5.2	−4.8	−6.2	−5.7	−6.9	−5.9
5	6-Octadecenoic acid	−4.8	−3.8	−3.8	−4.5	−5.3	−6.4	−5.7
6	Tetradecanoic acid	−5.4	−3.9	−3.6	−5.2	−5.1	−6.1	−5.6
7	n-hexadecanoic acid	−5.1	−4.0	−3.9	−4.9	−5.0	−6.5	−5.3
8	Butanoic acid ethyl ester	−4.2	−3.4	−3.2	−3.9	−3.7	−4.2	−4.1
9	Hexadecanoic acid ethyl ester	−6.1	−5.0	−4.7	−5.5	−5.3	−6.9	−6.0
10	Phytol	−5.8	−4.1	−4.5	−5.7	−5.5	−7.3	−6.3
11	n-heneicosane	−6.1	−4.3	−5.2	−5.6	−5.2	−7.4	−6.8
12	Butanoic acid, 2-amino-3-hydroxyl; propyl ester	−4.6	−3.9	−3.9	−4.6	−4.9	−4.7	−4.9
13	2,4,7-trinitrofluorenone	−6.7	−6.1	−6.0	−7.3	−6.9	−7.7	−5.4
14	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy	−5.5	−4.6	−5.0	−6.3	−5.8	−6.7	−6.3
15	9,12,15-octadecatrienoic acid	−6.2	−4.3	−3.8	−5.7	−5.9	−7.2	−5.9
16	9,12,15-octadecatrien-1-ol	−5.8	−3.7	−4.7	−4.8	−5.5	−6.5	−5.9
17	9,12-octadecadienoic acid	−6.3	−4.6	−4.9	−5.1	−5.5	−6.9	−5.4
18	Hexadecanoic acid methyl ester	−5.3	−3.9	−3.9	−4.8	−4.9	−6.2	−5.4
19	Cyclopentane undecanoic acid	−5.9	−4.2	−4.5	−5.6	−5.5	−6.1	−6.2
20	N-cyclododecyl acetamide	−6.4	−4.8	−5.3	−6.5	−7.0	−7.8	−5.1
21	Vernonioside E	−10.9	−8.7	−9.6	−11.0	−9.9	−11.1	−9.0
22	Luteolin	−7.4	−6.3	−6.9	−7.4	−8.1	−9.4	−6.4
23	Luteolin 7-*O*-beta glucoside	−9.9	−7.4	−8.6	−9.9	−10.4	−8.9	−9.9
24	Vernolide	−10.2	−7.4	−8.1	−9.8	−9.6	−10.7	−9.6
25	Vernolepin	−7.1	−5.7	−6.4	−7.5	−7.2	−8.9	−5.5
26	Vernodalin	−7.2	−6.1	−6.3	−7.4	−7.4	−8.6	−5.4
27	Vernomygdin	−7.1	−6.0	−6.7	−7.7	−7.7	−7.8	−5.1
28	Hydroxyvernolide	−6.8	−6.1	−7.0	−8.1	−8.0	−7.8	−6.1
29	Vernodalol	−6.9	−5.7	−6.4	−7.5	−7.2	−8.9	−5.4
30	Vernonioside D	−12.6	−10.7	−10.5	−11.8	−11.3	−13.2	−10.0
31	**MSG**	**−4.5**	**−4.5**	**−4.5**	**−4.9**	**−4.8**	**−5.1**	−5.0
32	**Ascorbic acid**					−5.5	−5.5	−4.5

Note: MSG and Ascorbic acid were used as standards.

As seen from the table, vernonioside D gave the highest docking scores for all the proteins tested. The highest binding energy obtained with the anti-apoptotic protein (Bcl-2) was given by Vernonioside D, closely followed by vernonioside E, vernolide and Luteolin 7-*O*-beta glucoside as seen from their relatively high ΔG values, while vernonioside D had the highest binding energy with the NF-kB protein. The interaction of Vernonioside D with the anti-apoptotic proteins is depicted in Fig. 2 (a, b).

Vernonioside D, Vernonioside E, luteolin 7-*O*-beta glucoside and vernolide gave relatively high binding affinities with the two repair proteins (DNA polymerase and ligase). Fig. 3 (a, b) depicts the interaction of Vernonioside D (compound with the greatest docking score) with DNA repair enzymes. Also, Vernonioside D, Vernonioside E, luteolin 7-*O*-beta glucoside and vernolide gave

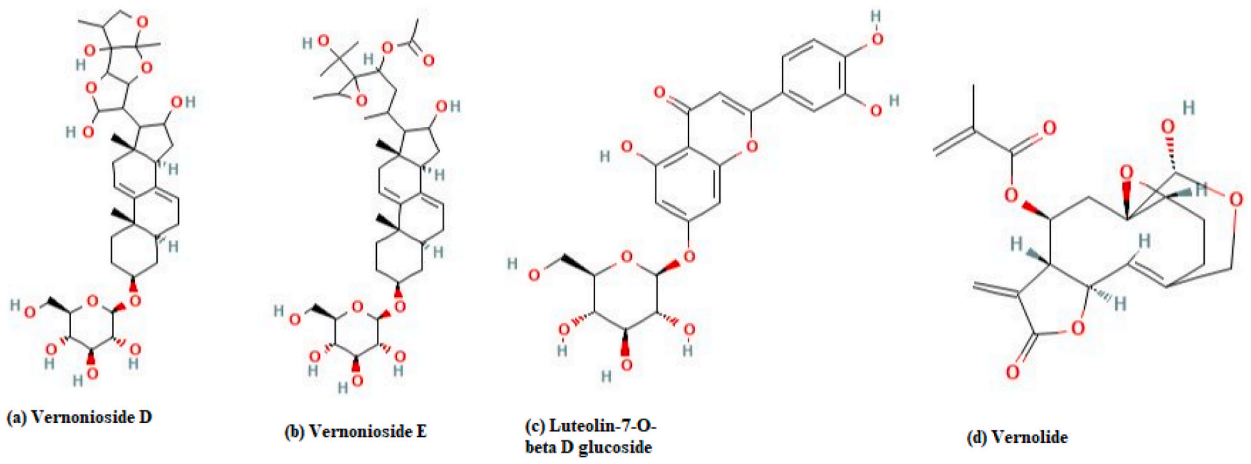


Fig. 1. Structures of lead compounds in *V. amygdalina* leaf with high affinities for anti-apoptotic proteins, DNA repair enzymes and antioxidant enzymes.

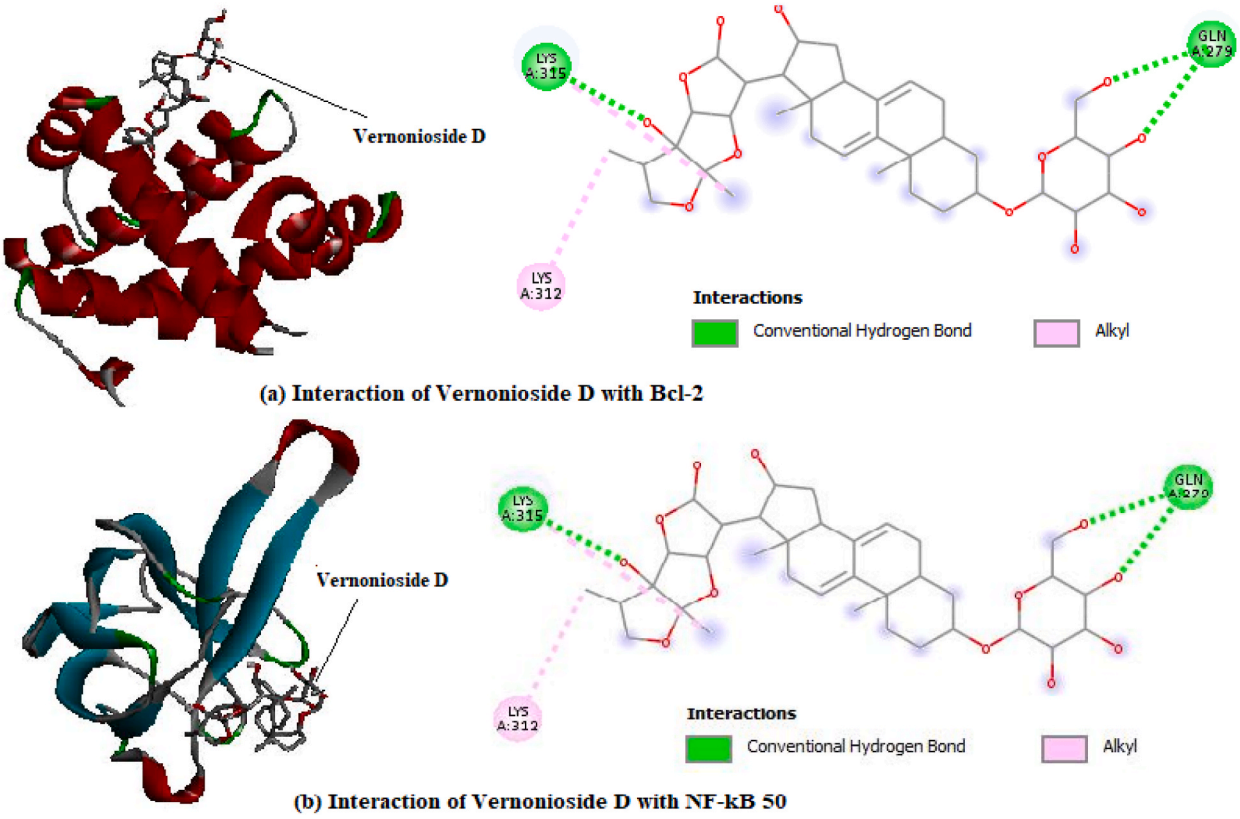


Fig. 2. (a, b): Interaction of Vernonioside D (compound with the greatest docking score) with anti-apoptotic proteins.

relatively high docking scores with the three antioxidant enzymes with the highest score given by vernonioside D and its interaction with antioxidant enzymes is shown in Fig. 4(a–c).

As seen from Fig. 2 (a, b), 3 (a, b) and 4 (a-c), the major interactions between the lead compound (vernonioside D) and the proteins are majorly that of conventional hydrogen bond, pi-alkyl bond, alkyl bond and carbon hydrogen bond.

3.3. Anti-genotoxicity test and white blood cell count determination

3.3.1. Anti-genotoxicity test

The result of the *anti*-genotoxic effect of *V. amygdalina* leaf is depicted in Table 4. As shown in the table, a significantly ($p < 0.05$) higher number of micronucleated cells were obtained from the blood of the animals when administered MSG. However, there was a reduction of about 18.4 % in the micronucleated cells when the animals were treated with *V. amygdalina* leaf extract.

3.3.2. Effect of V. amygdalina leaf extract on the neutrophil, lymphocyte and eosinophil counts in the experimental animals

Table 5 shows the effect of *V. amygdalina* leaf extract on the neutrophil, lymphocyte and eosinophil counts in the experimental animals. As seen from the table, there was a reduction in the neutrophil and eosinophil counts of the group treated with *V. amygdalina* leaf extract compared to the MSG only group. However, there was an increase in the lymphocyte count of the group treated with *V. amygdalina* leaf extract when juxtaposed with the MSG only group.

4. Discussion

Results obtained from the analysis of the phytochemical compounds in aqueous extract of *V. amygdalina* leaf revealed the presence of alkaloids, flavonoids, saponins, phenol and tannins with alkaloids being the most abundant. This is in tandem with the results obtained by Ref. [29] though there are variations which might be attributed to geographical, climatic and soil factors. However, [30], reported that the ethanolic extract of *V. amygdalina* contained more saponins and flavonoids. It is known that alcoholic extracts are able to extract lipophilic molecules better that aqueous extracts, especially phenolic compounds [31]. Bioactive compounds in plants are also referred to as phytochemicals. They are non-nutritional in nature but play roles in defending and preventing humans from diseases [32]. Flavonoids have been reported to demonstrate anti-inflammatory, anticancer properties in many studies, and above all, significant free radicals scavenging and antioxidant properties [33–35]. Tannins have been reported to inhibit inflammation,

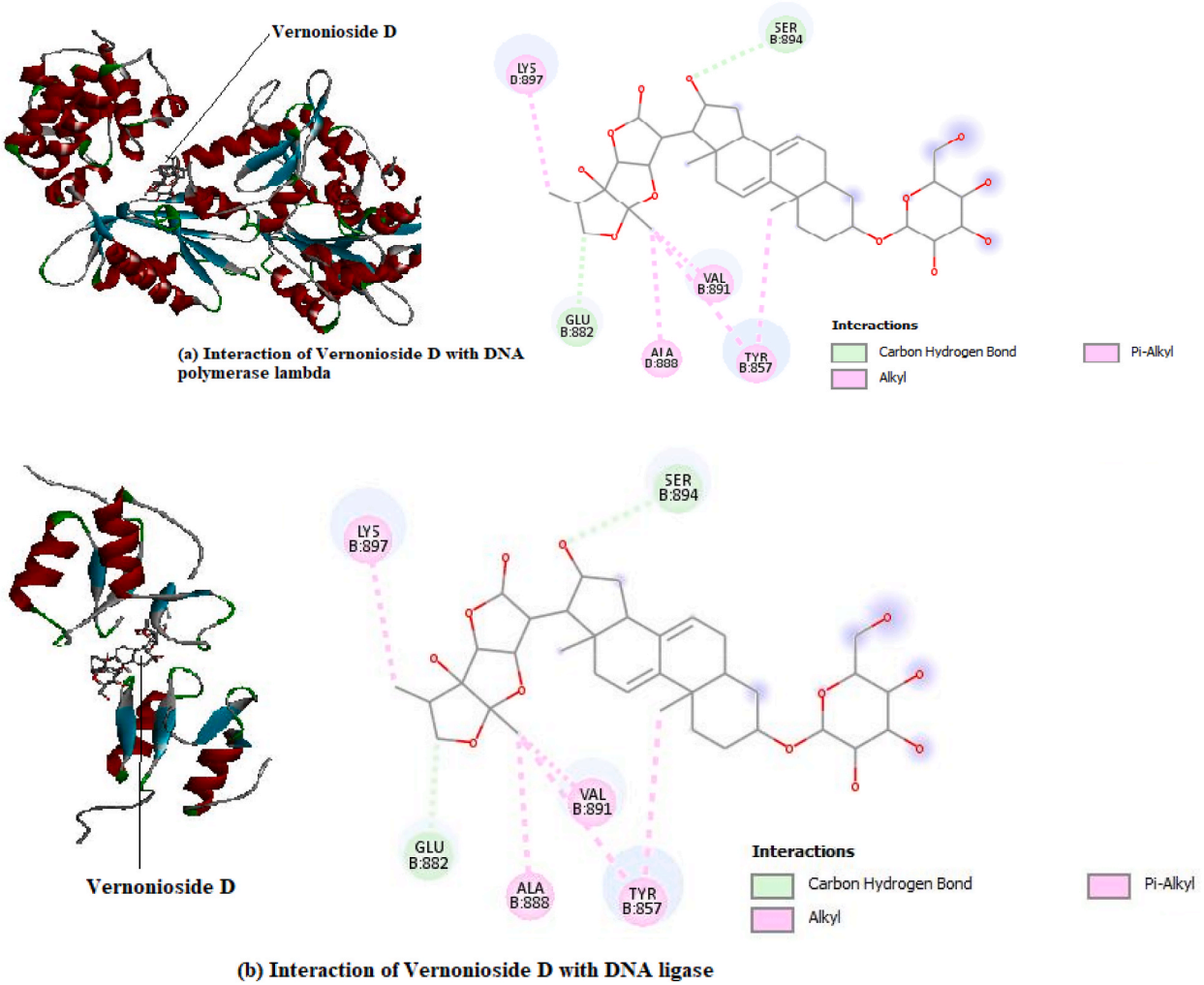
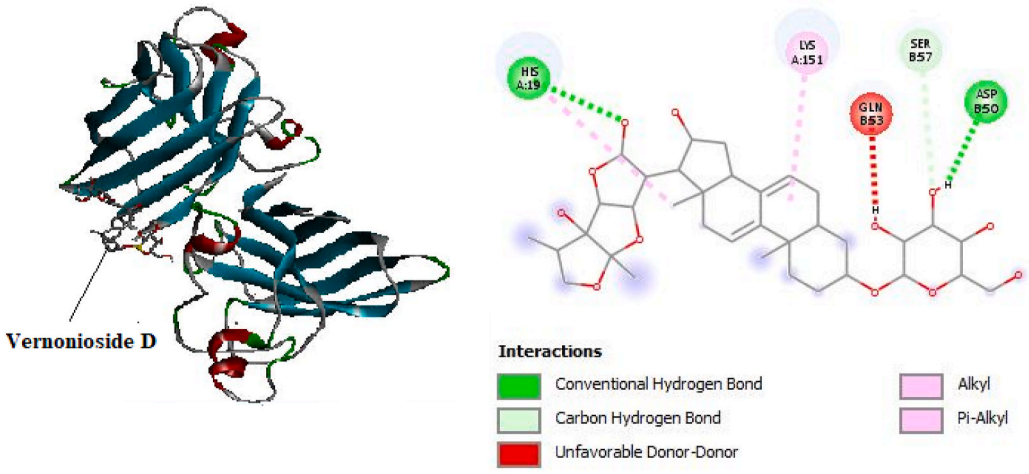


Fig. 3. (a, b): Interaction of Vernonioside D (compound with the greatest docking score) with DNA repair enzymes.

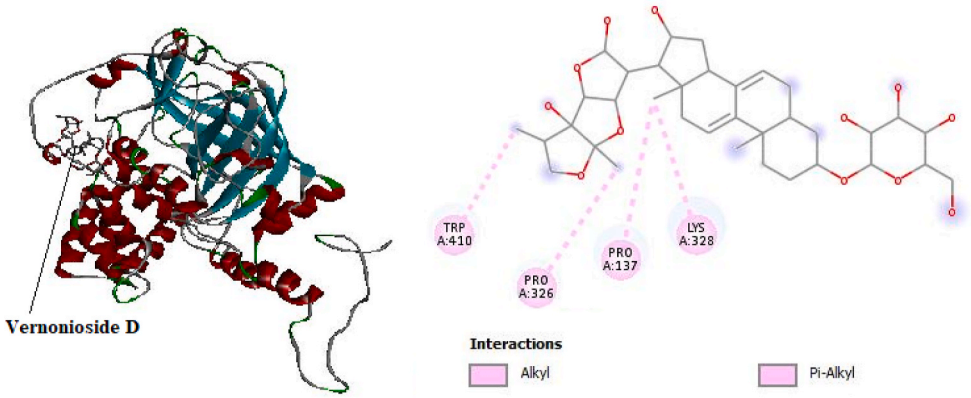
carcinogenesis and septic processes in humans [36]. Furthermore, tannins have also been shown to exert immune-modulating effects in earlier studies [37]. Alkaloids are substances with an extensive array of biological activities such as antiviral, antibacterial, anti-inflammatory and anticancer effects [38]. [39] reported that alkaloids extracts from *V. amygdalina* was able to scavenge free radicals in rats' penile tissues in a dose-dependent manner. Saponins are a group of compounds that can form complex with cholesterol, thereby reducing cholesterol levels in the body [40]. Saponins exhibit anti-mutagenic, anti-tumor and anti-inflammatory activities [41,42]. Phenols are also reported to protect against aging and carcinogens [43].

It has been proposed that MSG exhibits its genotoxicity through direct attack on the nuclear bodies within cells by triggering reactive oxygen species production in a way that it will overwhelm the body defence system such that there will be decrease in the level of antioxidant enzymes. Also, MSG-induced ROS production can result in programmed cell death (apoptosis) and changes in the genetic material [44]. Furthermore, glutamate receptors are known to be key players in the progression of MSG-induced disorders. Numerous types of glutamate receptors are expressed by T lymphocytes and these receptors regulate responses of the immune system [45,46]. The activity of voltage-gated potassium channels is altered by glutamate which subsequently leads to increase in the levels of calcium within the cell [47] which might result in excess calcium entering the mitochondria, and as such, the discharge of pro-apoptotic factors and a rise in reactive oxygen species production [48].

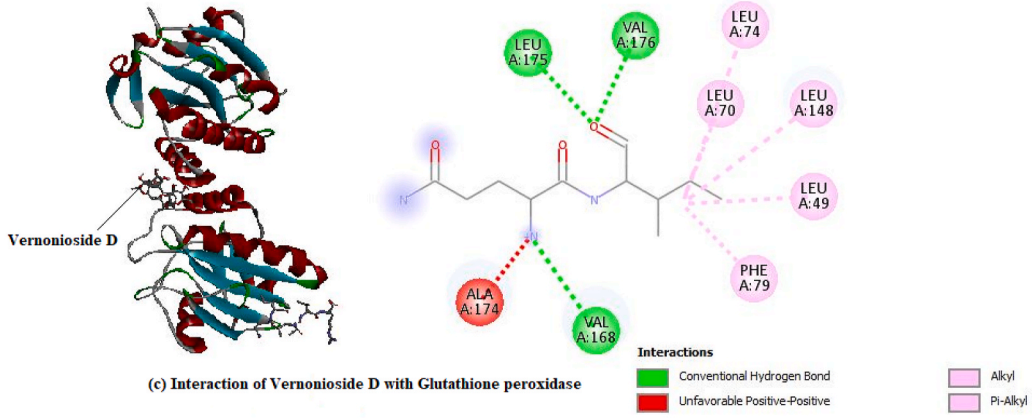
Results from molecular docking showed that vernonioside D, Vernonioside E, vernolide and luteolin 7-*O*-beta glucoside had higher binding affinities for the anti-apoptotic proteins, DNA repair proteins and the antioxidant enzymes than MSG used as the positive control. Bcl-2 is a member in the family of Bcl-2 proteins that help to preserve the integrity of the external membrane of the mitochondria by impeding the activities of the pro-apoptotic proteins [49]. Environmental stress is known to trigger NF-κB signaling pathway in which p50 becomes attached to NF-κB to form NF-κB p50 which acts to inhibit cell apoptosis [50,51]. A new enzyme that is responsible for DNA repair and meiotic rearrangement in human cells is DNA polymerase lambda [52]. DNA ligase is an enzyme that maintains the intactness of the genetic material by sealing breaks in the phosphodiester backbone of DNA and also correcting damage



(a) Interaction of Vernonioside D with Superoxide dismutase



(b) Interaction of Vernonioside D with Catalase



(c) Interaction of Vernonioside D with Glutathione peroxidase

Fig. 4. (a–c): Interaction of Vernonioside D (compound with the highest docking score) with antioxidant enzymes.

done to the DNA molecule [53]. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are enzymes that act as first line defense against reactive oxygen species [54].

Luteolin 7-*O*-beta glucoside is a flavonoid present in *V. amygdalina* leaves that has antioxidant effects [55]. Vernolide is a

Table 4
Evaluation of *anti*-genotoxic effect of *V. amygdalina* leaf extract on MSG-induced genotoxicity by the determination of the number of micronucleated erythrocytes.

Group	Micronucleated red blood cells
Control	2.00 ± 0.00
MSG only (4 g/kg)	4.50 ± 0.50*
MSG (4 g/kg) + *V. amygdalina* extract (250 mg/kg)	3.67 ± 1.56

Values are Mean ± SD of triplicate determinations, values with asterisk* are significantly different from the control at $p < 0.05$.

Table 5
Effect of *V. amygdalina* leaf extract on the neutrophil, lymphocyte and eosinophil counts in the experimental animals.

Group	Neutrophil	Lymphocyte	Eosinophil
Control	35.67 ± 1.11	64.00 ± 1.33	1.00 ± 0.00
MSG only (4 g/kg)	35.00 ± 1.00	61.00 ± 1.00	4.00 ± 0.00**
MSG (4 g/kg) + *V. amygdalina* extract (250 mg/kg)	32.75 ± 3.75	64.25 ± 2.88	3.80 ± 1.00**

Values are expressed as mean ± standard deviation of triplicate determinations, values with asterisks** are significantly different from control at $p < 0.05$, values with alphabetical superscripts are significantly different from MSG only at $p < 0.05$.

sesquiterpene lactone with antimicrobial, antioxidant and antitumoral activities [56,57]. Vernoniosides D and E are steroid glycosides with anti-helminthic and anti-inflammatory properties [58,59]. Exposure to MSG led to a statistically significant increase in the number of micronucleated cells in the animals which confirmed the genotoxicity of MSG and its capability of damaging the nuclear arrangement of host cells as corroborated by Refs. [60,61]. However, administration of *V. amygdalina* extract led to a reduction in the number of micronucleated cells thereby suggesting the *anti*-genotoxic effects of the extract. [62] also reported the protective effect of the aqueous extract of *V. amygdalina* against DNA damage *in vitro*. It can be inferred that the *anti*-genotoxic effect of *V. amygdalina* leaf extract might be attributed to specific bioactive compounds which showed high binding affinities for the anti-apoptotic proteins and the DNA repair enzymes as confirmed by the docking studies. The synergistic interactions of these compounds might have led to the activation of the anti-apoptotic proteins and the DNA repair enzymes which ameliorated the genotoxic effects of MSG by counteracting its DNA damaging effect. The occurrence of flavonoids and phenolic compounds in the extract could have an impact on the antioxidant effect which is also a mechanism by which the extract ameliorated the genotoxic effect of MSG as these compounds are also known to stimulate the production of antioxidant enzymes [63] which can be adduced to the high binding affinities for the enzymes as corroborated by the *in silico* studies.

MSG also caused a reduction in the neutrophil and lymphocyte count while resulting in an increase in the eosinophil count. MSG is known to possess immunosuppressive effects due to its ability to cause disturbances in production of blood cells [64,65]. Administration of *V. amygdalina* extract resulted in a decrease in both the neutrophil and eosinophil counts in the animals exposed to MSG but led to an increase in the lymphocyte count of the animals. Studies by Refs. [66,67] corroborated the immune stimulating property of the aqueous extract of the plant. The major type of leukocytes that plays a role in short-term response to inflammation are neutrophils. A reduction in the neutrophil and eosinophil count of the treated animals suggests the anti-inflammatory activity of the extract against MSG-induced genotoxicity as MSG has been implicated in oxidative stress-induced inflammation [68]. The reduction in the neutrophil count might be attributed to alkaloids, flavonoids, saponins and other compounds like vernoniosides D and E which are present in the extract which are known to possess anti-inflammatory effects. Lymphocytes are white blood cells that defend the body against infections and diseases by eliminating pathogens [69]. The mechanism might be linked to the various bioactive compounds in the extract especially those with antioxidant effects since antioxidants are alluded to be involved in preserving the ability of immune cells to fight against oxidative stress which can result in a compromised immune status [70].

5. Conclusion

In conclusion, the aqueous leaf extract of *Vernonia amygdalina* showed immune boosting and ameliorative properties against MSG-induced genotoxicity in rats. This might be attributed to the synergistic interaction of some of the bioactive compounds acting to inhibit apoptosis and to stimulate DNA repair enzymes and antioxidant enzymes as confirmed by *in silico* studies. However, further studies is recommended on the isolation, characterization and biological activities of vernoniosides D and E which showed the highest binding affinity with all the proteins used in this study as they represent novel lead compounds which could be utilized in the prophylaxis and treatment of oxidative stress and its attendant complications.

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Data availability statement

No data associated with this study has been deposited into a publicly available repository. Data included in article/supp. Material/ referenced in article.

CRediT authorship contribution statement

Temitope Abiola: Writing - review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Emmanuel O. John:** Writing - original draft, Resources, Investigation, Formal analysis, Data curation. **Ibukun Temitope Sossou:** Validation, Resources, Project administration, Investigation. **Benedict Charles Callistus:** Visualization, Validation, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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