Research Article

Induction of Metastatic Cervical Cancer via HeLa Cell Xenograft in Female Wistar Rats: Modulatory Effects of Yoyo Bitters

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Abstract

Yoyo Bitters (YYB) is a herbal formulation reported to reduce cellular free radical damage, remove harmful toxins, etc. Here, we report the investigation of the therapeutic activity of YYB on HeLa cell xenografted rats. Thirty rats were distributed into: control, dexamethasone, HeLa, HeLa + dose (0.2 mL/kg YYB), and HeLa + dose2 (0.6 mL/kg YYB) groups. Following immunosuppression, the rats were xenografted with HeLa cells to induce metastatic cervical cancer. The rats were administered YYB daily for two weeks. Whole blood was obtained for haematological analyses (total white blood cells (WBC), lymphocytes (LYM), neutrophils (NEUT), platelets (PLT), mid-range absolute (MID) counts) and to determine total protein (TP) levels. Serum activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione *S*-transferase (GST), and levels of reduced glutathione (GSH), lipid peroxidation (LPO) and nitric oxide (NO) were determined spectrophotometrically. The expression levels of vimentin, cytokeratin, Bax and Bcl-2 proteins were determined immunohistochemically. HeLa cell xenografts caused a significant decrease in the levels of WBC, LYM and PLT, but the levels of MID and NEUT increased significantly ($p < 0.05$). YYB modulated WBC, LYM, PLT, MID, and NEUT to levels that are not significantly ($p > 0.05$) different from the negative control. Furthermore, YYB dose-dependently modulated the activities of SOD, CAT and GST. In addition, the significantly induced levels of LPO and NO in HeLa cell xenograft group were abated by YYB. Immunohistochemically, YYB modulated the levels of all proteins assessed with an inclination toward apoptosis. Yoyo Bitters showed potential therapeutic activity via the modulation of antioxidant status.

Key Words: *cervical cancer; HeLa cells; immunohistochemistry; immunosuppression; metastasis; Yoyo Bitters*

INTRODUCTION

In 2019, the Global Cancer Observatory (GLOBOCAN) estimated over 570, 000 new cervical cancer cases and 311, 000 cervical cancer-related deaths (Global Cancer Observatory, 2019). In women, it was the most occurring cancer, after breast cancer, colon cancer and lung cancer, and it was also the fourth most common cause of cancer mortality among women (Arbyn *et al*., 2020). The highest frequency of occurrence of cervical cancer was evaluated in Eswatini (Swaziland), with approximately 6.5% of women having cervical cancer cases before 75 years of age. Around the world, the age of cervical cancer detection was 53 years, spanning 44 to 68 years (Arbyn *et al*., 2020). Among other ones, the risk factors of cervical neoplasm include: inadequate screening, multiple sex partners, young age at intercourse, obesity, tobacco use, hormonal contraceptives use, human papillomaviruses (HPVs) infection particularly HPV-16 and HPV-18 (Kashyap *et al*., 2019).

Traditional herbal medicine and their preparations have been widely used for thousands of years not just in developing countries but also in developed ones owing to their natural origins and lesser side effects (Kamboj, 2012). These herbal medicines initially took the forms of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Majaz and Khurshid, 2016). Hitherto, herbal medicine remains the mainstay of about 75–80% of the world population, principally in the developing countries, for primary health care (Kamboj, 2012). One of the local herbal products produced by an indigenous health care provider in Nigeria is Yoyo Bitters.

Yoyo Bitters (YYB) is a plant-based medication introduced into the Nigerian market in 2003. It was certified by the National Agency for Food, Drugs Administration and Control (NAFDAC) as a herbal medication without liquor, colouring or counterfeit additives (Shugaba *et al*., 2014). Since its presentation, the polyherbal preparation has acquired wide acknowledgement and use by many people (Shugaba *et al*., 2014). The main phytoconstituents of YYB include: *Aloe vera*,

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Acinos arvensis, Citrus aurantifolia, Chenopodium murale and *Cinamomum aromaticum.* The identified components of YYB, based on phytochemical analyses, are: alkaloids, amino acids, flavonoids, glycosides/reducing sugar, proteins, saponins, tannins, terpenoids and minerals (Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn), as captured together in Figure 1 (Anionye and Onyeneke, 2016). Previously, we have reported the gas chromatography–mass spectrometry (GC-MS) analysis of YYB carried out using GC – MSD 5973 Agilent instrument following column chromatographic separation of the product. Furthermore, structure elucidations of the compounds were subsequently performed using nuclear magnetic resonance and fourier-transform infrared spectroscopy (Onyeaghala *et al.*, 2015a). Among the compounds isolated are: 2-cyclohexen-1-ol, 4-amino 5,6 dimethoxy, furyl hydroxymethyl ketone, 1,2,3,4,5-cyclopentanepental, etc.

The herbal mixture has been noted for its ability to mitigate cellular damage associated with exposure to free radicals and facilitate the elimination of toxins from the body. Consequently, YYB can boost the body's immune system against ambient and opportunistic infections (Oyewo *et al.*, 2013; Shugaba *et al.*, 2014). Using human cervical cancer cell, Onyeaghala *et al.* (2015b) investigated the cytotoxicity of fractions obtained from YYB and they demonstrated that it possessed potential anticancer activities. The experiments of the current study were designed to investigate the effect of YYB on HeLa cells xenograft metastatic model in female Wistar rats.
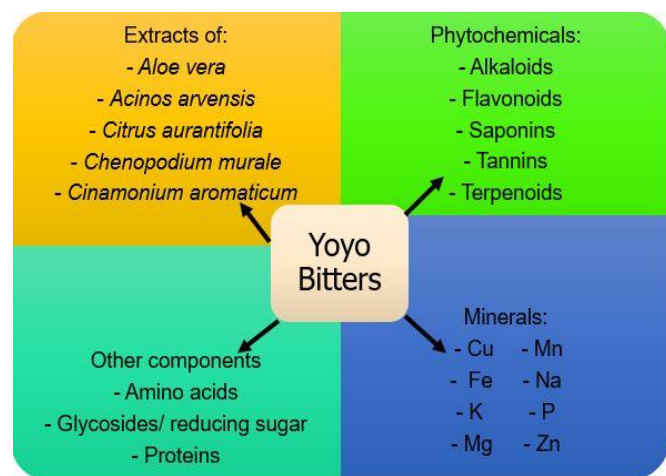

Figure 1:
Pictorial representation of the main phytochemical constituents and other components of Yoyo Bitters (YYB) (Anionye and Onyeneke, 2016)

MATERIALS AND METHODS

Chemicals and reagents for analysis: Yoyo Bitters (NAFDAC No.: A7-1051L) and dexamethasone were purchased from Kunle-Ara Pharmaceuticals, Ibadan, Nigeria. Other reagents including: dipotassium hydrogen phosphate trihydrate, thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5′, 5′-dithiobis-2-nitrobenzoic acid (DTNB), 1-choro-2, 4-dinitrobenzene (CDNB), glutathione (GSH) and epinephrine were of analytical grade.

Cell culture: HeLa cell line was obtained from the Department of Virology, College of Medicine, University of Ibadan. The cells were grown in Eagle's minimum essential (MEM, Sigma-Aldrich, MO, USA) medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) L-glutamine, 100 U penicillin and 100 μg/mL streptomycin at 37 ℃ in a humidified atmosphere with 5% CO_2. The cells were passaged twice weekly. Cell viability was also ascertained based on Trypan Blue dye exclusion method (Rosengard and Cochrane, 1983) using a Neubauer chamber of a haemocytometer to count cells.

Experimental animals: Thirty (30) non-gravid female Wistar rats weighing between 130 ± 10 g were obtained and housed in the Animal Colony of the Department of Biochemistry, College of Medicine, University of Ibadan. The animals were kept in clean cages with a temperature of 26 ± 2 °C and they were allowed to acclimatize for two weeks under standard laboratory conditions with free access to feed pellets and clean water throughout the experiment. This study was carried out following the protocol of laboratory Animal Care and Use Research Committee (ACUREC), University of Ibadan, concerning guiding principles for biomedical research involving animals. Before the commencement of the study, ethical approval (UI-ACUREC/19/0027) was obtained.

Administration of Yoyo Bitters: To ascertain the anticancer potentials of YYB, two different doses were administered: Dose 1 (0.2 mL/kg) and dose 2 (0.6 mL/kg), based previous publication and average daily human consumption (Oyewo *et al.*, 2013). The administrations were carried out orally for 14 days (2 weeks). The negative control group was given distilled water which was used as the vehicle for the drug (Table 1).

Table 1:
Experimental design.

Group	Treatment
1	Healthy control
2	20 mg/kg bw dexamethasone (initial) + 10 mg/kg bw dexamethasone booster dose weekly.
3	Positive control - (One microlitre (1 μL) of 5 x 10^6 HeLa cells + dexamethasone administered weekly)
4	Positive control + Yoyo Bitters (dose 1, YYB1) administered daily
5	Positive control + Yoyo Bitters (dose 2, YYB2) administered daily

After a two (2)-week period of immunosuppression using dexamethasone, as previously publications, HeLa cells were injected at the tail region targeting the caudal/tail vein (Arjomandnejad *et al.*, 2014; Omotosho *et al.*, 2021).

Determination of mean body weight: The body weight of each rat was taken using a digital chemical balance (Laboratory Scale SF-400) before and after the experimental period (as initial and final body weight, respectively), and the mean body weight correspondingly computed. Weight changes were expressed as percentage weight increase.
Percentage weight increase was calculated from the formula:

$$\frac{Wy - Wx}{Wy} \times 100$$

Where, Wx = initial mean total body weight; Wy = final mean total body weight.

Termination of experiment: Twenty-four (24) hours after the last treatment of the animals, they were sacrificed. Thereafter, blood and lung samples were collected. The blood samples were collected in both plain and ethylenediaminetetraacetate bottles. Portions of the lung samples were fixed in 10% formalin.

Tissue homogenate preparation: The excised lung samples of the experimental animals were rinsed in 1.15% KCl and collected into already labelled sample bottles on ice. The samples were then weighed and homogenised in 0.1 M phosphate buffer pH 7.4. The homogenates were centrifuged at 12,000 rpm for 15 min to obtain the post mitochondrial fraction. The supernatant of each homogenate was collected into properly labelled bottle and subsequently used for biochemical assays.

Estimation of haematological parameters: Using whole blood samples, haematological parameters (total white blood cells (WBC), lymphocytes (LYM), neutrophils (NEUT), platelets (PLT), mid-range absolute (MID) counts) were estimated with AMP Accos 360 haematology analyzer manufactured by AMEDA Labordiagnostik GmbH, Austria. The principle is based on the coulter counter method that accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended, in a conductive liquid (diluent), acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume and the particular analyte measured (Don, 2003).

Biochemical assays:
Determination of total protein: Protein concentrations of the various homogenates were determined using the biuret method as described by Gornall *et al.* (1949).

Determination of superoxide dismutase (SOD) activity: The activity of SOD in the serum was determined by the method of Misra and Fridovich (1972).

Determination of catalase (CAT) activity: Catalase activity was determined according to the method of Claiborne, (1985).

Estimation of glutathione peroxidase (GPx) activity: Glutathione peroxidase activity was measured according to the procedure of Rotruck *et al.* (1973).

Estimation of glutathione-S-transferase (GST) activity: Glutathione-S-transferase activity was determined according to Habig *et al.* (1974).

Estimation of reduced glutathione (GSH) level: The method of Beutler (1975) was followed in estimating the levels of reduced glutathione (GSH).

Assessment of lipid peroxidation (LPO): Lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) present in the test sample according to the method of Wall *et al.* (1976).

Determination of nitric oxide (NO) level: The levels of NO were determined using the Griess reagent, as described by Giustarini *et al.* (2008).

Immunohistochemical analyses: The fixed lung samples were used for immunohistochemical assessments. Lungs tissues of the best representative of each group were stained immunohistochemically for cytokeratin AE1 and AE3, and vimentin. Sections were also stained with antibodies against Bcl-2 and Bax to assess apoptosis.

Statistical analysis of results: The results were expressed as mean ± standard error of the mean (SEM). Differences between the groups were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with GraphPad Prism 6.0. P-values <0.05 were considered statistically significant for differences in means

RESULTS

Metastatic cervical cancer in HeLa cell xenografted rats resulted in weight loss: Figure 2A shows the weight-change effect of treatment of female Wistar rats with HeLa cells based on metastatic cervical cancer model. As expected, the healthy control group recorded a positive increase in the body weight contrary to the HeLa cell xenografted rats which displayed a significant ($p < 0.05$) decrease in body weight. In one of the HeLa cell-treatment groups, YYB caused an increase in the body weight as compared with the group exposed to HeLa cells alone, although the influence of YYB on body weights was not significant. Also, the rats in dexamethasone group were able to recover their initial average body weight before the termination of the work

Modulatory influence of YYB on haematological parameters in cervical cancer metastatic model of immunocompromised rats: Across board, the HeLa cell-xenografted animals recorded a significant ($p < 0.05$) decrease in total white blood cell (WBC) counts which were not restored by treatment with YYB (Figure 2B). Furthermore, the trend displayed by lymphocyte counts followed that of WBC (Figure 2C). The introduction of HeLa cell alone resulted in a significant increase in neutrophil counts as compared with the negative control group (Figure 2D). Platelet counts almost followed the same trend as lymphocytes with YYB having a boosting effect, although not significantly as compared with the HeLa cell-alone treatment group (Figure 2E). Lastly, higher dose of YYB (dose 2) significantly ($p < 0.05$) reduced the HeLa cell- and dexamethasone-induced mid-range absolute count (MID) to almost the same value as the healthy control group (Figure 2F).
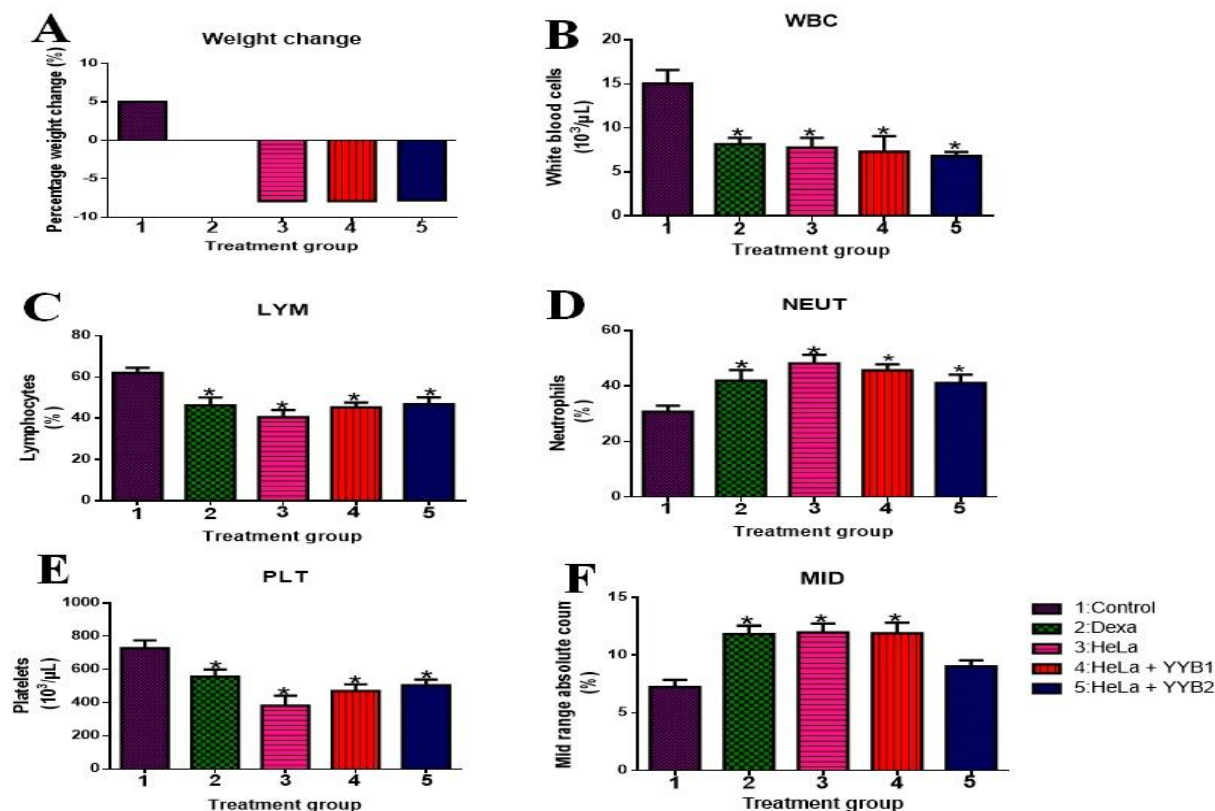
Figure 2:
Changes in the body weights (A) and influence of YYB on haematological parameters (B-F) in cervical cancer metastatic model of immunocompromised female Wistar rats. These charts show changes in the levels of total white blood cells (WBC), lymphocytes (LYM), neutrophils (NEUT), platelets (PLT) and mid-range count (MID). Results are represented as Mean ± SEM (n = 6). *- Significantly (p < 0.05) different from negative control group.
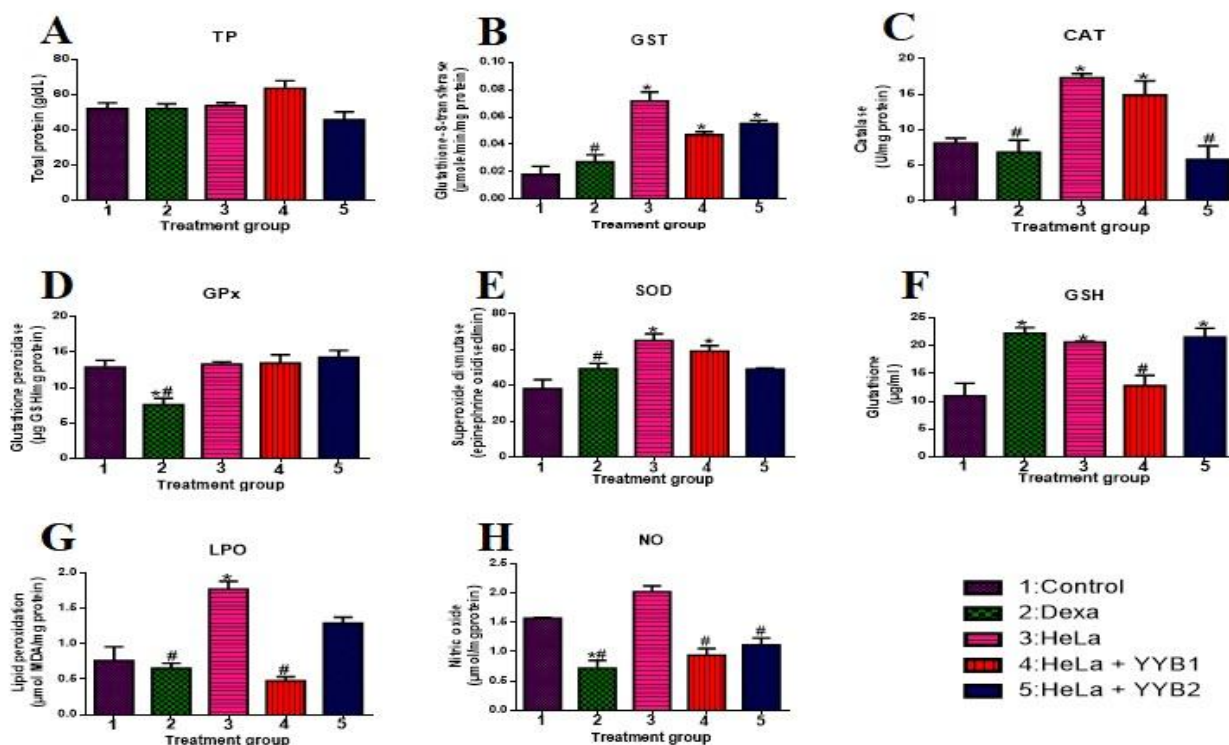

Figure 3:
Modulatory roles of YYB on total serum protein levels (A), changes in the activities of some enzymatic antioxidants (B-E), levels of reduced glutathione (F), lipid peroxidation (G), and nitric oxide (H) in the lungs of immunocompromised female Wistar rats xenografted with HeLa

cells. Results are represented as Mean ± SEM (n = 6). *- Significantly different from healthy control group, #- significantly different from HeLa-alone group

HeLa cells and dexamethasone did not significantly modulate the serum total protein levels, but YYB did: Figure 3A shows that the administration of YYB modulated the serum levels of total proteins in rats xenografted with HeLa cells. Both HeLa cells alone and dexamethasone alone did not induce a significant ($p < 0.05$) change in serum protein levels as compared with the healthy control group. Overall, YYB displayed a dose-dependent decrease in serum total protein levels, albeit, non-significantly ($p < 0.05$).

Yoyo Bitters modulated the activities of antioxidant enzymes in the lungs of immunocompromised rats xenografted with HeLa cells: We observed that the metastatic cervical cancer model is associated with a significant ($p < 0.05$) increase in the activities of catalase, glutathione *S*-transferase and superoxide dismutase in the lungs of the experimental animals. In addition, the activities of these enzymes were significantly modulated by YYB when compared with the healthy control group (Figure 3B, C, E). However, only the dexamethasone-treated group displayed a significant decrease in the activities of glutathione peroxidase, while there is no significant change in all the other groups as compared with the healthy control group (Figure 3D).

HeLa cells and YYB significantly increased the levels of reduced glutathione in the lungs of immunocompromised female Wistar rats: In comparison with the healthy control group, the levels of GSH were significantly ($p < 0.05$) increased by HeLa cells and YYB, in a dose-dependent manner (Figure 3F).

Ameliorative effect of YYB on lipid peroxidation in the lungs of immunocompromised HeLa cell-treated female Wistar rats: We recorded a significant increase in the levels of lipid peroxidation in the lungs of dexamethasone-induced immunosuppressed rats that were xenografted with HeLa cells. However, the co-administration of different doses of YYB resulted in a significant ($p < 0.05$) decrease in the levels of lipid peroxidation when compared with the HeLa cell only, immunosuppressed group (Figure 3G).

Immunocompromised female Wistar rats xenografted with HeLa cells expressed a significant level of nitric oxide (NO) in the lungs, but YYB reduced it: Treatment of female Wistar rats with dexamethasone alone resulted in a significantly reduced level of NO in the lungs while xenografting these animals with HeLa cells caused a significant rise in NO levels. However, the administration of YYB mitigated the effect of HeLa cells (Figure 3H).

Immunohistochemical expression of vimentin in the lungs of immunosuppressed female Wistar rats xenografted with HeLa cells: As shown in Figure 4, the YYB treatment reduced the expression of vimentin as compared with the HeLa cell xenografted group. The effect of YYB was dose-dependent with the higher dose down-regulating the expression of vimentin almost to the level obtained for the healthy control group.
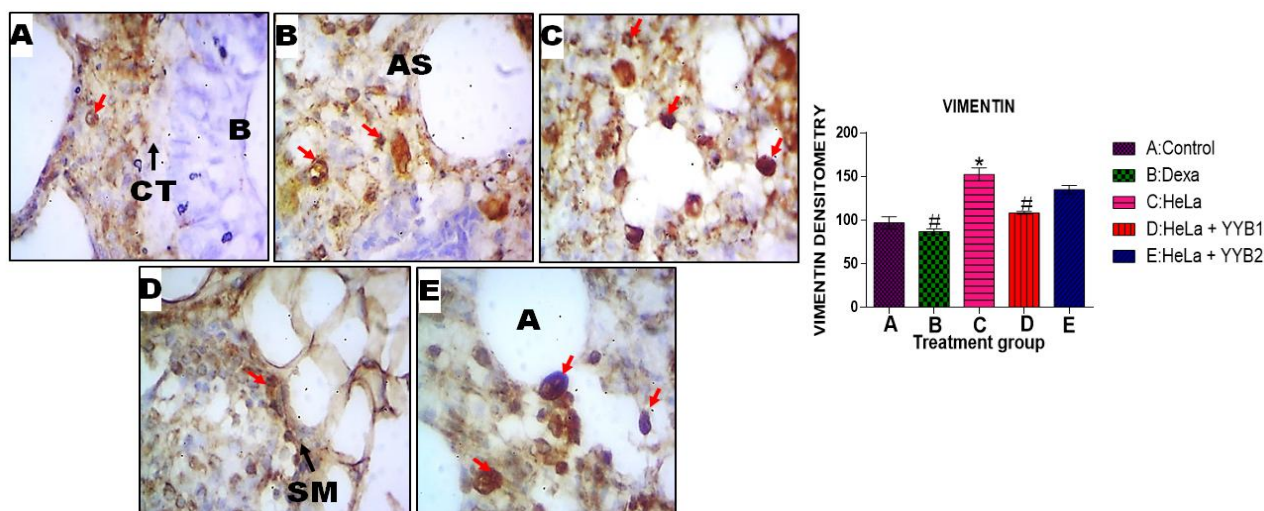

Figure 4:
Immunohistochemical photomicrographs indicating the expression of vimentin in lung samples of immunosuppressed female Wistar rats xenografted with HeLa cells and treated with YYB in comparison with the healthy control group (Mag.: x100). Empirical changes in the levels of vimentin among the healthy, positive, and intervention groups are displayed in the bar chart.

Immunohistochemical expression of cytokeratin in the lungs of immunosuppressed female Wistar rats xenografted with HeLa cells: Based on Figure 5, the expression of cytokeratin was detected immunohistochemically in all the groups except the dexamethasone-treated group. The YYB treatment of the immunosuppressed female Wistar rats xenografted with HeLa cells resulted in a remarkable decrease in the expression of cytokeratin when compared with the healthy control and HeLa cell xenografted groups.

Immunohistochemical expression of Bax protein in the lungs of immunosuppressed female Wistar rats xenografted with HeLa cells: Bax protein was most remarkably expressed in the healthy control as compared with all the other treatment groups (Figure 6). However, the lower

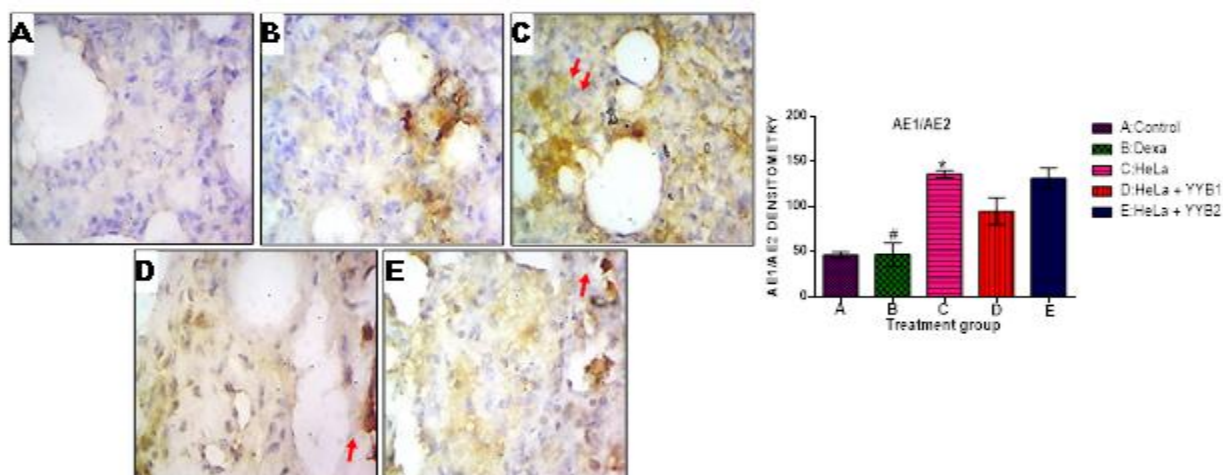dose of YYB was able to induce the expression of Bax remarkably.

Figure 5:
Immunohistochemical photomicrographs indicating the expression of cytokeratin in lung samples of immunosuppressed female Wistar rats xenografted with HeLa cells and treated with YYB in comparison with the healthy control group (Mag.: x100). Empirical changes in the levels of cytokeratin among the healthy, positive, and intervention groups are displayed in the bar chart.
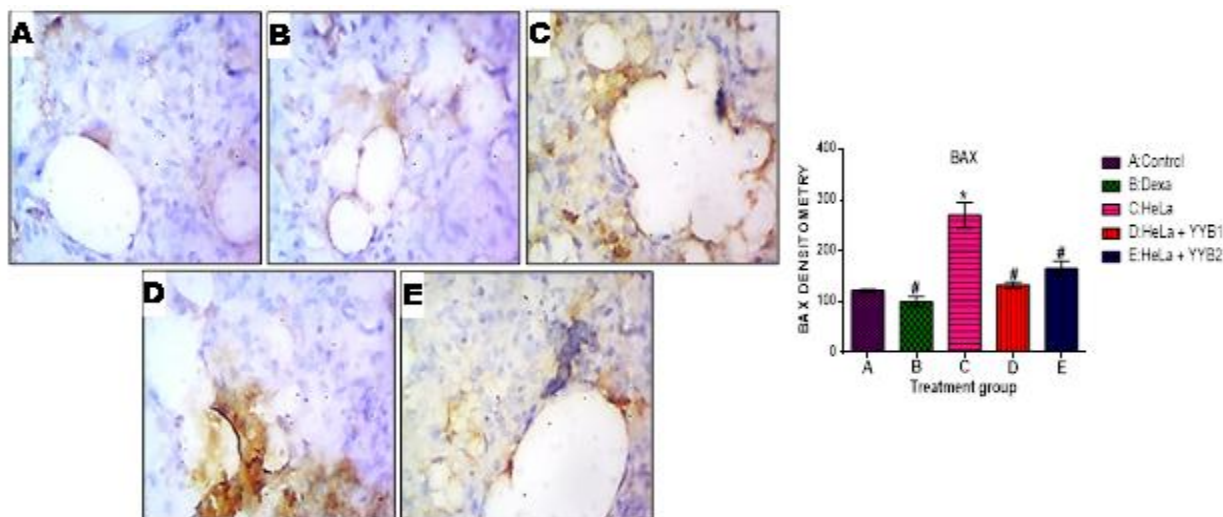

Figure 6:
Immunohistochemical photomicrographs indicating the expression of Bax proteins in lung samples of immunosuppressed female Wistar rats xenografted with HeLa cells and treated with YYB in comparison with the healthy control group (Mag.: x100). Empirical changes in the levels of Bax proteins among the healthy, positive, and intervention groups are displayed in the bar chart.
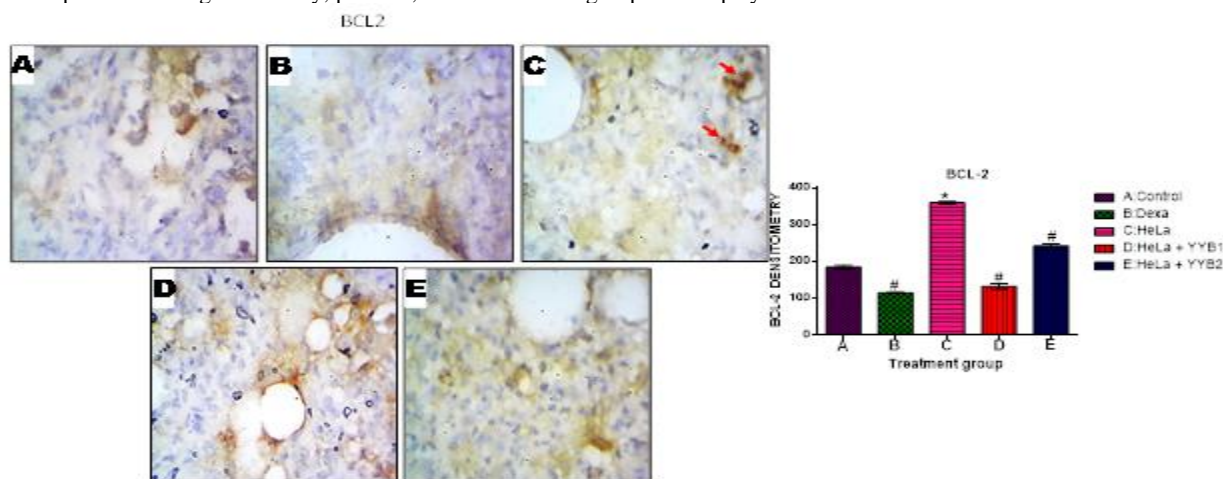

Figure 7:

Immunohistochemical photomicrographs indicating the expression of Bcl-2 proteins in lung samples of immunosuppressed female Wistar rats xenografted with HeLa cells and treated with YYB in comparison with the healthy control group (Mag.: x100). Empirical changes in the levels of Bcl-2 proteins among the healthy, positive, and intervention groups are displayed in the bar chart.

Immunohistochemical expression of Bcl-2 proteins in the lungs of immunosuppressed female Wistar rats xenografted with HeLa cells: Although the expression of Bcl-2 proteins in the immunosuppressed group xenografted with HeLa cells was higher, but it was not significantly different from that of the healthy control. However, treatment of the xenografted Wistar rats with different doses of YYB resulted in a remarkable decline in the expression of Bcl-2 proteins (Figure 7).

DISCUSSION

Early stage of cervical cancer is normally managed through surgery, as radical hysterectomy is a significant choice (Liu *et al*., 2017). After surgery, follow-up treatment choices include radiotherapy, chemotherapy, etc. to forestall tumour metastasis or recurrence. However, for patients with advanced cervical cancer, the viability of the above options is very low, and the side effects may be deleterious (Abu-Rustum *et al*., 2001) Plant-based anticancer drugs have great potentials in drug design (Gbadegesin *et al*., 2017; Olugbami *et al*., 2020). For instance, safe and efficient new anticancer drugs have been obtained from plants, including paclitaxel, camptothecin, docetaxel, etc. (Skwarczynski *et al*., 2006; Venditto and Simanek, 2010). In the current study, we investigated the therapeutic potentials of a plant-based medication, Yoyo Bitters (YYB), in the management of metastatic cervical cancer model induced by HeLa cells in female Wistar rats.

The immunosuppressed rats xenografted with HeLa cells all experienced weight loss, which may be due to the increased metabolism induced by the presence of cancer cells. Cancer cells depend principally on glucose for their metabolism and stored glycogen in the skeletal muscles and liver can be degraded into glucose molecules. The sustained breakdown of glycogen may account for the weight loss reported (Kreitzman *et al*., 1992).

We recorded a significant increase in the levels of neutrophils on exposure of the experimental animals to dexamethasone, as already reported (Anafi *et al*., 2014). Xenografting the immunosuppressed animals with HeLa cells led to a further increase in neutrophil levels. This may be an immune response mechanism in an attempt to eliminate the exogenous cells. However, on treatment with YYB, the levels of neutrophils significantly declined. The implication here is the possibility of YYB to control the excess influx of immune cells to a target site associated with prolonged inflammation. It is known that increased peripheral neutrophil and decreased lymphocyte counts reflect an enhanced tumour proliferation (Ponchel *et al*., 2005). Furthermore, we observed that treatment with YYB modulated the levels of other haematological parameters in comparison with the healthy control group which may boost immunity against the cancer cells. Serum total protein level, which is a reflection of albumin and globulin levels, was not significantly altered across the various groups.

Among other features, oxidative stress is associated with cancer initiation and progression (Reuter *et al*., 2010). Hence, we investigated the influence of YYB on the antioxidant status. The activities of SOD, an enzymatic antioxidant, have been reported to become elevated in patients with advanced cervical cancer (Jelić *et al*., 2018). In this work, we observed an elevation in the activities of SOD due to HeLa cell xenograft, but the administration of YYB resulted in a remarkable decline in activities. This may be due to the mopping up of superoxide radicals by YYB (Liu *et al*., 2018). Catalase is another enzymatic antioxidant; an elevation in the activities of this enzyme has been reported in cervical cancer patients, especially at advanced stages (Jelić *et al*., 2018). Its function, in this case, may be a physiological response to counter the levels of hydrogen peroxide to normalise the oxidative stress caused by the cancer cells (Ng *et al*., 2007). We observed the modulatory role of YYB on catalase activities with both groups administered with YYB displaying lower activities than the untreated HeLa cell xenograft group. Furthermore, the activities of GPx were not significantly (p < 0.05) different among the various groups except for the dexamethasone-treated group, which recorded a significant decrease in activities. This report is also similar to that of Liu *et al*. (2018) which showed that gandoderic acids could decrease GPx levels in HeLa cells, thereby inducing oxidative stress and, subsequently, apoptosis. We also considered the activities of lung GST; the significant increase in its activities may be linked to an attempt to relieve the oxidative stress caused by HeLa cells that have migrated to the lungs. However, treatment with the different doses of YYB abrogated the raised activities of GST. We also assessed the levels of reduced glutathione, a non-enzymatic antioxidant. HeLa cell xenograft caused an increase in lung GSH levels, while on treatment with YYB, we recorded a further increase in the levels of GSH in one of the YYB-treated groups as compared with the HeLa cell xenograft group. It should also be noted that at the lower dose of YYB, there was a decline in GSH levels, which is still within the safe range as obtained for the healthy control. However, the ability of YYB to significantly reduce the GSH levels as compared with the xenograft group may be an indication that, depending on the dosage, administration of YYB can result in GSH depletion to a level that can induce apoptosis (Liu *et al*., 2015)

In this study, a significant elevation in the levels of LPO was observed in the lungs as a result of HeLa cell xenograft. This is in concordance with the report of Jelić *et al*. (2018) associating cervical cancer patients with a significant increase in the levels of LPO. However, the administration of YYB mitigated the oxidative stress-induced LPO.

Nitric oxide is a marker of inflammation and it serves as an essential antioxidant by reacting rapidly with peroxyl radicals (Hummel *et al*., 2006) Upon treatment with YYB, the levels of nitric oxide, which had been exacerbated in the lungs of rats xenografted with HeLa cells, were significantly reduced as compared with the healthy control. Therefore, it can be inferred that YYB possesses anti-inflammatory activity.

Epithelial-mesenchymal transition (EMT) features epithelial cells losing their extremities/bonds, and gaining

developmental properties, making them exhibit more of the features of mesenchymal cells. Epithelial-mesenchymal transition results in loss of epithelial markers, such as E-cadherin, claudin, occludin, plakophillin, cytokeratin and desmoplakins, and addition of mesenchymal markers, for example, vimentin, SNAI, N-cadherin, Zeb1, and Zeb2 (Qureshi *et al.*, 2015). Based on immunohistochemical analysis, vimentin is most upregulated in the lungs of animals xenografted with HeLa cells. It is implied that EMT had already been initiated within the period of treatment and this has the tendencies of enabling the spread of cancer cells. However, upon treatment with YYB, we observed a reduction in vimentin expression levels. This indicates that YYB administration may be able to delay or mitigate the spread of cervical cancer by downregulating vimentin expressions. Furthermore, the dexamethasone-treated animals showed that the expression levels of vimentin were downregulated even below what obtained for the healthy control. Cytokeratin AE1/AE3 gradually decreases in expression as EMT progresses (Filipovic *et al.*, 2017). However, there was a slight elevation in its expression upon HeLa cell xenograft and subsequent reduction in the same upon YYB administration.

HeLa cell xenograft in rats resulted in a decrease in Bax expression levels, but the administration of YYB resulted in an increase in its expression. Furthermore, HeLa cell xenograft caused an increase in Bcl-2 expression, but YYB administration resulted in a decline in its expression. From these results, the ratio of Bax to Bcl-2 occasioned by YYB favours apoptosis thereby increasing the amount of cancer cell death in the lungs (Onyeaghala *et al.*, 2015b).

CONCLUSION
Our findings indicate the potentials of YYB to boost immunity, initiate an oxidative-stress-induced apoptosis, and inhibition of EMT via the modulation of cytokeratin and vimentin expression levels. The YYB may therefore possess anticancer properties.

Abbreviations: CAT, catalase; GPx, glutathione peroxidase; IHC, immunohistochemistry; LPO, lipid peroxidation; LYM, lymphocytes; GSH, reduced glutathione; MDA malondialdehyde; MID; mid-range absolute count; NEUT, neutrophils; NO, nitric oxide; PLT, platelets; TP, total protein; SOD, superoxide dismutase; WBC, total white blood cells; YYB, Yoyo Bitters.

Author contributions: OAO: Study design, supervision of the study design and manuscript writing. IOO: Study design, supervision of the study design and manuscript writing. MAG: Study design, supervision of the study design and manuscript writing. JOO: Study design, supervision of the study design, involved in performing the experiments, drafted and fine-tuned the manuscript. AMA: Study design, supervision of the study design, and fine-tuned the manuscript. OAA: Correction of study design, performed the experiments, acquisition and analysis of data, and editing of manuscript. ISM: Performed the experiments, acquisition and analysis of data, and partially involved in editing of manuscript. CAA: Performed the experiments, acquisition and analysis of data. All authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

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