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Quail (*Coturnix japonica*) egg attenuated 2-butoxyethanol-induced enzymatic dysregulation, disseminated thrombosis and hemolytic impairment in female wistar rats

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ABSTRACT

Influence of quail egg on pathologies has increased research interests and series of investigations are currently being done on its influence against these pathologies. The influence of quail egg against 2-butoxyethanol induced hemolysis and disseminated thrombosis was investigated to determine the enzymatic regulations that ensue in the amelioration of deleterious hemolytic and disseminated thrombosis displayed in female Wistar rats. Quail egg was separated into three (3) components (extracts)-quail egg yolk water soluble (QYWS) and fat soluble (QYFS), and albumen extract (QA) and the inorganic and organic compositions were characterized. Depranocytotic assaults was achieved by 250 mg/kg of 2-Butoxyethanol administered for 4 days, the clinical observation revealed a dark purple-red discoloration on the distal tails of the rats and therapeutic applications followed with 1000 mg/kg BWT of QYWS, QYFS and QA, and 15 mg/kg BWT of hydroxyurea. Morphological evaluation, haematological estimations and biochemical evaluations of the influence on the activities of sphingosine kinase-1, RNase, red cell carbonic anhydrase, lactate dehydrogenase, glutathione peroxidase and caspase-3, vis a vis the concentrations of sphingosine-1 phosphate, selenium and zinc (plasma and urine). *In vitro* anti-inflammatory influence of quail egg components were investigated against hemolysis and key enzymes of inflammation-cycloxygenase, lipoxygenase and β-glucuronidase. The *in vitro* anti-inflammatory effects of QYWS, QYFS and QA were concentration dependent from 200 to 800 μg/ml against hemolysis and the key enzymes of inflammation. The characterization of inorganic and organic bioactive composition of the yolk and albumen revealed the presence of folic acid, cobalamin, pyridine, riboflavin, ascorbic acid as well as vitamins D and E, selenium, zinc, iron and calcium. These had reflected in the attenuation of the induced hemolytic and disseminated thrombosis by regulations of enzymes linked to the infarction, apoptosis and oxidative stress characterized in sickle cell index.

1. Introduction

2-Butoxyethanol (BE) is an environmental toxicant used as chemicals in the manufacturing of wide range of domestic and industrial products as well as surface coatings and household cleaning agents (Lewis et al., 2005). BE was reported to be metabolized in Fischer 344 female rats to 2-butoxyacetic acid (BAA) causing acute hemolytic anemia (Ezov et al., 2002) and disseminated thrombosis. This 2-butoxyethanol-induced acute disseminated thrombosis and infarction in female rats may be caused by vaso-occlusion, possibly initiated by acute hemolytic anemia with the possibility of release of procoagulant factors from destroyed erythrocytes, altered morphology and decreased deformability of erythrocytes, and a tendency of the red blood cells to aggregate and/or adhere to the endothelium (Ghanayem and Sullivan, 1993; Koshkaryev et al., 2003; Nyska et al., 2003).

Depranocytosis also known as sickle cell disease is a genetic disorder due to the substitution of the glutamate by valine at the sixth position of the β-chain of the hemoglobin A (HbA). This causes a structural modification that alters oxygen affinity and transport of the oxygenated sickled RBC leading to tissue infarction vis a vis hemolytic anemia and disseminated thrombosis.

Unfortunately, current therapies are very limited and less efficient. Bone marrow transplant is the most promising therapy, but it is too expensive for most Africans which are most affected by this genetic

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disorder (Mpiana et al., 2013). Anti-sickling agents such as hydroxyurea and decitibine have been developed and their modes of actions are essentially pathophysiological, by inhibiting HBS polymerization and RBCs sickling process and to protect sickle RBCs from oxidatively induced damages (Mpiana et al., 2013). Hydroxyurea has also been shown to decrease the number and severity of sickle cell crises by increasing fetal hemoglobin production significantly in patients with sickle cell anemia (Cokic et al., 2003). Other clinical anti-sickling agents are tellurite, thiocyanate and folic acid had been clinically validated. Administration of folic acid, vitamins B6 and B12 were also utilized to reduce the episodes of chronic anaemia in sickle cell patients. Patients taking the vitamins-folic acid, B6 and B12 had significant improvement in their pack cell volume (PCV) but blood abnormalities and impaired growth rate associated with the disease were not improved (Ohnishi et al., 2000). Inorganic nutritional supplements such as iron and polyunsaturated fatty acids in fish oil supplements were also reported to reduce the severity of anaemia (Ohnishi et al., 2000). However laboratory tests showed that the anti-anaemic effect was slight and considered insignificant (Redding-Lallinger, 2006).

Clinical research ideas evolved from the quest to develop an alternative therapies which would have both clinical and nutritional acceptability and most importantly affordable, compared to unavailable orthodox drugs which had partial acceptability as a result of their side effects against hepatic, renal and neurological functions (Oyewole et al., 2008).

Quail (*Coturnix japonica*) egg have been administered via different routes of administrations such as oral, dermal applications and other therapeutic routes to evaluate the pharmacognostic applications (Tunsaringkarn, 2012). Acceptance in Nigeria was as a result of folkloric belief that the egg produced by natural means was active in the management of diabetes mellitus, sickle cell anemia and degenerations emanating from apoptosis and oxidative stress progression. Quail egg helps to relieve anaemia by increasing the level of haemoglobin in the body while removing toxins and heavy metals (Tunsaringkarn, 2012). The Japanese quail egg have being claimed to improve metabolism, combat stress, helps in the treatment of obesity, asthma and various forms of allergies (Truffier, 1978). Based on these findings, an experimental method that will mimic RBCs occlusion, tissue infarction, anemia and disseminated thrombosis through 2-butoxyethanol was hypothesized and the influence of quail egg constituents was investigated against key markers of inflammation, apoptosis, oxidative stress and enzymatic dysregulations.

2. Methodology

2-Butoxyethanol of > 99% purity was purchased from Sigma-Aldrich Chemical Co. (Rehovot, Israel). Quail eggs were purchased from a poultry farm at Ita-Ogbolu Local Government Area, Ondo State. Nigeria. Eggs were gently broken and the contents were poured into a petri-dish. The yolk was carefully separated from the albumen. And both were stored at 4 °C until used.

2.1. Extraction of quail egg constituents

2.1.1. Quail egg yolk water soluble (QYWS) and fat soluble (QYFS) constituents

QYWS fraction was collected by accurately weighing 100 g of the dried yolk powder into 1000 ml volumetric flasks and 800 ml of distilled water was added. The mixture was heated in a shaking water bathe for 24 h at 50 °C. The heated mixture was centrifuged in cold centrifuge at 4000 g for 10 min. Supernatant was collected, freeze-dried and stored at 4 °C.

QYFS constituents from the quail egg yolk was extracted using Ibukun and Oladipo (2016) method. 100 g of yolk was dissolved in chloroform (400 mL) in a test tube fitted with a condenser, and 1% sulfuric acid in methanol (200 mL) was added, mixture was vortexed for 2 h and 50 mL of sodium chloride solution (5%w/v) was added, and the required esters was extracted with 50 mL of hexane. Pasteur pipettes was used to separate the layers. The hexane layer was washed with 50mLof 2% w/v potassium bicarbonate solution and dried over anhydrous sodium sulfate. The solution was harvested to remove the drying agent, and the solvent removed under reduced pressure in a rotary film evaporator. The fat soluble extract was stored in an air-tight container in a desiccator at 4◦C until needed.

2.1.2. Quail egg albumen constituents

The hydrolysis of albumen and extraction was carried out by mixing equal volume of albumen and mixture of 0.1% trifluoroacetic acid and 6 M Hydrochloric acid solution in the ratio 1:2. The mixture was incubated at 100 °C for 24 h. The combined extracts were evaporated to dryness in an evaporator and the residues were dissolved in 25 µL of 0.25 M borate buffer, pH 8.8 (Weiss et al., 2007).

2.2. Chromatographic determination of components

The analyses of amino acids and vitamin content were carried out using HPLC device. Prior to injection into the HPLC (Agilent 1200 series) the solutions were filtered through a 0.2 µm filter (Millex-GN), HPLC was coupled with a detector (Agilent 1260), column (Chromspher 5, C18) at temperature 40 °C, the dimension was 5 µm, 3 mm × 250 mm with Hamilton microliter syringe. The flow rate was 0.7 ml/min.

Mineral elements were determined as described. Samples were oven dried before ashing at 550 °C for 5 h. Addition of 20 mL of 1 N hydrochloric acid (HCl) followed by qualitative and quantitative analysis by Inductively Couple Plasma (ICP) for phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn) and nitrogen (N) analysis.

2.3. Treatments

Female Wistar albino rats (120 ± 20 g) procured from a private animals holding facility in Ogbomoso, Nigeria were used for the *in vivo* study. The rats were fed on standard laboratory diet, given water *ad libitum* and maintained under laboratory conditions. All of the animals received human care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the Centre for Research and Development, Federal University of Technology, Akure, Nigeria. The experiment was carried out at the Applied Clinical Biochemistry Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Nigeria.

A dose of 250 mg/kg of 2-Butoxyethanol administered for 4 days was revealed in previous experiments (Ezov et al., 2002) to be the most appropriate dose to produce disseminated thrombosis and used with slight modification. The treatments with the daily single dose quail egg components commenced 18 h after the assault and lasted for 30 days.

Group 1: Sham operated with oral administration of 0.9% normal saline solution,
Group 2: BE induction (2-Butoxyethanol (250 mg/kg/day for 4 days)) and 1000 mg/kg BWT of QYWS extract,
Group 3: BE induction (2-Butoxyethanol (250 mg/kg/day for 4 days)) and 1000 mg/kg BWT of QYFS extract,
Group 4: BE induction (2-Butoxyethanol (250 mg/kg/day for 4 days)) and 1000 mg/kg BWT of QA extract,
Group 5: BE induction (2-Butoxyethanol (250 mg/kg/day for 4 days)) and 15 mg/kg BWT of Hydroxyurea treatment and
Group 6: BE induction (2-Butoxyethanol (250 mg/kg/day for 4 days)) control with oral administration of 0.9% normal saline solution.

2.4. Necropsy, blood collection and preparation

Eighteen hours after the last administration, rats were sacrificed by cervical dislocation. Blood samples were collected by cardiac puncture into non-anticoagulant serum tubes and allowed to stand for 1 h. The clotted blood was centrifuged at 4 °C for 10 min at 3000 g. The serum was transferred into clean tubes for the evaluation of biochemical indices.

2.5. Morphological examination

Emmel's test and Hemolysis test were performed as previously reported (Mpiana et al., 2010). The RBCs digitize micrographs were treated with a computer assisted image analysis system.

2.6. Evaluation of heamatological parameters

Hematocrit (PCV) was determined using high-speed centrifugation of blood-filled hematocrit tubes with a Zipocrit Hematocrit Centrifuge (ThermoFisher Scientific, Philadelphia, PA). All white blood cell (WBC) count estimates were performed by the same techniques, at a location on the slide where the cells were one layer thick, adjacent to one another (membranes touching), evenly distributed, and showed no signs of morphological changes (Newman and Piatt, 1997). White blood cell estimates were made by using a 100× objective lens with immersion oil, counting the number of white blood cells in 10 fields, calculating the average, and then multiplying the number of cells by 2000. The absolute cell count for each type of cell was calculated by multiplying the percentage of the type of cell by the overall WBC estimate (Newman and Piatt, 1997). Total erythrocyte count (TEC) and total leukocyte count (TLC) were determined by Haemocytometric method. With the use of haemocytometric diluting fluid, RBCs were viewed with transparent cytoplasm and pale staining nucleus. Immediately after the collection of blood, smears were prepared for differentiating and counting each type of leukocytes. Differential count of leukocytes was determined by using Giemsa stain. The smears were stained approximately 2–10 min after methyl alcohol fixation. Two hundred leukocytes, including granulocytes and agranulocytes were counted in different fields of the smears and expressed in percentage (%).

The Mean Corpuscular Hemoglobin (MCH) value is determined by the equation: MCH = (HGB/RBC) x 10.

The MCHC is classically determined by the equation: MCHC = (HGB/HCT) x 100.

The MCV is classically determined by the equation: MCV = (PCV/RBC).

Determination of SPHK1 activity and protein level in the erythrocytes of mice.

Erythrocyte pellets were lysed in a buffer containing 20 mM PIPES, 150 mM NaCl, 1 mM EGTA, 1% v/v Triton X-100, 1.5 mM $MgCl_2$, and 1 mM Na-orthovanadate (pH 7.4). The total protein concentration was measured with a Protein Assay Kit (Bio-Rad).

Determination of RNase and Red Cell Carbonic Anhydrase Activities.

RNase (EC 3.1.4,22) activity in plasma was assayed by a method reported previously (Prasad and Oberleas, 1973).

Red cell carbonic anhydrase (EC 4.2 d.1) protein was measured by a radioimmunosorbent technique (Magid et al., 1973).

2.7. Determination of plasma and urine zinc concentration

Plasma and urine Zinc was assayed with an atomic absorption spectrophotometer (Model 303; PerkinElmer Corp., Norwalk, Conn. 06856) according to methods published earlier (Prasad et al., 1965). Plasma was diluted fourfold and assayed directly by using standards in glycerol:water (5:95 by vol). Undiluted samples of urine were assayed directly by atomic absorption spectrophotometry. Phosphates in the urine do not interefere with zinc assay by atomic absorption spectrophotometry *(10)*. Washed erythrocytes were lysed with de-ionized water and then digested with concentrated HNO_3 before assay. Samples were washed with hexane and ethanol, dried overnight at 125 °C, and then digested with concentrated HNO_3 before assay.

2.8. Determination of serum lactate dehydrogenase activity

All analyses were carried out on non-hemolyzed blood specimens (Rotenberg et al., 1988). Samples drawn during the conditioning period were analyzed the same day they were drawn. Total serum LDH activity was determined by following the decrease in optical density at 340 nm in a reaction mixture containing 2.8 ml of 0.1 M potassium phosphate buffer, 0.2 mg NADH, 0.1 ml of a solution of 2.5 mg/ml sodium pyruvate and 0.1 ml serum. One unit of LDH activity will cause a decrease in optical density of 0.001 optical density units per minute at 340 nm. Sample incubation and cuvette compartments were maintained at 25 °C. The reaction was carried out for three minutes to insure a linear rate. Samples were diluted where necessary to obtain a linear rate. The linear decrease was expanded to four minutes and change in optical density per minute calculated. The following equation was used to calculate LDH activity:

Units of LDH per ml serum = (1000 x Abs per minute)/ml serum used.

2.9. Determination of glutathione peroxidase (GPx) activity

The reaction mixture containing final concentrations of 1.5 mM tris HCl pH 7, 10% Triton x-100 (peroxide free), 2 Mm NADPH, 1.5 mM GSH, 1 mM Glutathione Reductase, 5% NaN_3, and 100 µL cell lysate was initially pre-incubated at 370c. Initial absorbance was registered at 340 nm for 5 min at intervals of 1.5min. 10 mM H_20_2 was added and final absorbance reading was also registered at the same wavelength for an additional 10 min at intervals of 1.5min. GPx activity was calculated by the change in the absorbance value at 340 nm and expressed as micromoles of NADPH/minute/µg protein (Rahman et al., 2006).

2.10. Determination of blood selenium concentration

Selenium determination was carried out as described by Hamdy et al. (2015) using Atomic Absorption Spectrometer (Model 303; PerkinElmer Corp., Norwalk, Conn. 06856).

2.11. Determination of Caspase-3 activity

Sample	5X Assay Buffer	Caspase-3 Sample	Inhibitor	DI H2O	Substrate	Total Volume
Buffer Blank	10 µL	0 µL	0 µL	40 µL	0 µL	50 µL
Blank	10 µL	0 µL	0 µL	30 µL	10 µL	50 µL
Test		Sample	10 µL	10 µL	0 µL	20 µL
10 µL	50 µL					

Assay mixture was prepared according to the above table. About 40 µL of the assay mixture was added to a well plate. The reaction was initiated by adding 10 µL of substrate solution and incubated at 37 °C for 1 h. Ten (10) µl of the reaction mixture was transferred to another well plate suitable for reading in a luminometer at 405 nm. The plates were read for 5 s per well using a single 60 µl injection of freshly made Luciferase Substrate solution (Chemicon, 2002).

2.12. In vitro evaluation anti-inflammatory activities

2.12.1. Inhibition of albumin denaturation

Assay was carried out using Leelaprakash and Mohan (2011) model with slight modification. The reaction mixture consisted of test extracts

and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1 N HCl. The sample extracts were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, after cooling the samples the turbidity was measured at 660 nm. The percentage inhibition of protein denaturation was calculated as follows: Percentage inhibition =(Abs Control –Abs Sample) X 100/Abs control.

2.12.2. Inhibition of proteinase activity

Assay was carried out using Leelaprakash and Mohan (2011) model with slight modification. The reaction mixture (2 ml) contained 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations. The mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated. Percentage inhibition = (Abs control –Abs sample) X 100/Abs control.

2.12.3. Inhibition of heat-induced haemolysis

Assay was carried out using Leelaprakash and Mohan (2011) model with slight modification. The reaction mixture (2 ml) consisted of 1 ml test sample of different concentrations and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 °C for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm.

The experiment was performed in triplicates for all the test samples. The Percentage inhibition of Haemolysis was calculated as follows: Percentage inhibition = (Abs control –Abs sample) X 100/Abs control.

2.12.4. Inhibition of hypotonicity-induced haemolysis

Assay was carried out using Leelaprakash and Mohan (2011) model with slight modification. Different concentration of extract, reference sample, and control were separately mixed with 1 ml of phosphate buffer, 2 ml of hyposaline and 0.5 ml of HRBC suspension. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged at 3000 rpm. The supernatant liquid was decanted and the haemoglobin content was estimated by a spectrophotometer at 560 nm. The percentage hemolysis was estimated by assuming the haemolysis produced in the control as 100%.

Percentage protection = 100- (OD sample/OD control) x 100.

2.12.5. Inhibition of cyclooxygenase activity

The inhibition assay was carried out using Anoop and Bindu (2015) model with slight modification. The mixture contained Tris-HCl buffer, glutathione, hemoglobin & enzyme. The assay started by the addition of arachidonic acid and terminated after 20 min incubation at 37 °C by addition of 0.2 ml of 10% TCA in 1 N HCl, mixed and 0.2 ml of TBA was added and contents heated in a boiling water bath for 20 min, cooled and centrifuged at 1000 rpm for 3 min. The supernatant was measured at 632 nm for COX activity.

% inhibition = [{Abs control- Abs sample}/Abs control] x 100.

2.12.6. Inhibition of lipoxyginase activity

Anti-Lipoxygenase activity was studied using linoleic acid as substrate and lipoxidase as enzyme (Anoop and Bindu, 2015). Test samples were dissolved in 0.25 ml of 2 M borate buffer pH 9.0 and added 0.25 ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 25 °C. After which, 1.0 ml of linoleic acid solution (0.6 mM) was added, mixed well and absorbance was measured at 234 nm. The percent inhibition was calculated from the following equation.

% inhibition = [{Abs control- Abs sample}/Abs control] x 100.

2.12.7. Inhibition of β-glucuronidase activity

The β-glucuronidase activity was determined by spectrophotometry using an optical absorbance measurement of p-nitrophenol, formed from the substrate using the method of Collins et al. (1997), modified as follows: the reaction mixture made in a final volume of 100 µL contained 70 µL of 0.1 M acetate buffer pH 5.0 (3.402 g/250 mL of sodium acetate, pH was adjusted with 0.1 M acetic acid), 10 µL of test extracts, 10 µL of Enzyme (24 U) pre-incubated for 10 min, and 10 µL of 0.5 mM p-nitro phenyl-beta-D-glucuronide. The mix reaction was then incubated at 37 C for 30 min. The reaction was stopped by the addition of 50 µL of 0.2 M Na_2CO_3. After the reaction had been stopped, the optical absorbance was measured against the reagent blank value by spectrophotometry at 405 nm. The percentage of enzyme inhibition was calculated using the following equation:

Inhibition (%) = $[(A_0 - A_1) \times 100]/A_0$. Where A_0 = Control (total enzyme activity without inhibitor); A_1 = Test (activity in the presence of test compound).

2.13. Statistical analysis

The results were pooled and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) was used to analyze the results and Duncan multiple tests was used for the post hoc (DMRT). Statistical package for Social Science (SPSS) 17.0 for Windows was used for the analysis. The significance level was set at $p < 0.05$.

3. Results

Clinical confirmation of red-stained feaces after the second day of 2-butoxyethanol assault was recorded, there was also a dark coloration at the tip of the tail on the fourth day. None of the control animals had these clinical features.

3.1. Morphology of the hemolyzed blood

3.2. Summary of the morphology

Group 1: The erythrocyte presented biconcave normal form.
Group 2: The biconcave form of the erythrocyte was almost recovered.
Group 3: The biconcave normal form was almost recovered.
Group 4: The sickle shape of the erythrocyte was gradually reversed
Group 5: The biconcave normal form was almost recovered.
Group 6: The sickle form was seen.

The perimeter, surface and radius were calculated to determine the effects of QYFS, QYWS and QA as well as hydroxyl urea (HU) treatments in hemolysed and deformed RBCs in order to confirm the modification showed by the micrographies (Table 1). The result revealed that the average radius for the RBCs of the blood of 2-butoxyethanol treated animals could not be calculated; because hemolysed and deformed RBCs of untreated blood in the positive control group (Group 6) were not

Table 1
Influence of Quail egg yolk water and fat soluble constituents, and albumen components on physical cellular parameters of the blood of female albino rats.

	Cellular perimeters (µm)	Cellular surfaces (µm^2)	Cellular radius (µm)
Group 1	14.22 ± 2.31^a	30.39 ± 3.37^d	2.97 ± 0.42^d
Group 2	14.92 ± 1.09^a	26.45 ± 1.40^c	2.32 ± 0.92^b
Group 3	19.69 ± 2.11^b	22.43 ± 1.55^b	1.92 ± 1.03^a
Group 4	14.66 ± 0.95^a	23.9 ± 2.27^b	2.09 ± 0.88^a
Group 5	18.02 ± 1.06^b	24.7 ± 2.51^b	2.89 ± 0.58^c
Group 6	29.48 ± 2.36^c	19.29 ± 2.38^a	0

circular. The average radius appeared after treatment of sickle RBCs with the quail egg components indicating the re-appearance of the normal form of RBCs. Hydroxyurea treatments revealed the significantly highest cellular radius of 2.89 ± 0.58 μm, QYWS, QYFS and QA had significantly increased cellular radius which are 2.32 ± 0.92 μm, 1.92 ± 1.03 μm and 2.09 ± 0.88 μm respectively (see Fig. 1).

3.3. Evaluation of mechanism of inhibiting hemolysis and dessiminated thrombosis

The influence of 1000 mg/kg BWT of QYWS, QYFS and QA on sphingosine kinase-1 activity was investigated in the management of hemolysis and disseminated thrombosis arising from 2-butoxyethanol induction in Fig. 2. The activity of the enzyme was significantly inhibited by hydroxyurea to the level that was not significantly different compared to its activity in the animals in the negative control group ($p < 0.05$), but significantly different compared to the quail egg components treatments ($p < 0.05$). There was no significant difference in the SPHK1 activity in the animals treated with QYWS, QWFS and QA ($p < 0.05$), however there was a significant inhibition of the SPHK1 activity compared to estimations in blood of animals in the positive control group ($p < 0.05$). Fig. 3 showed the influence of the quail egg components and hydroxyurea on the concentration of sphingosine-1 phosphate after hemolysis and disseminated thrombosis arising from 2-butoxyethanol induction. Quail egg components- QYWS, QWFS and QA demonstrated a significant reducing effects on SPH-1-PH $p < 0.05$), however, hydroxyurea had significantly greater reducing influence on SPH-1-PH than the quail egg components $p < 0.05$).

The influence of 1000 mg/kg BWT of QYWS, QYFS and QA as well as hydroxyurea on the activity of RNase was investigated in the management of hemolysis and disseminated thrombosis arising from 2-butoxyethanol induction in Fig. 4. The activity of RNase was significantly reduced by hydroxyurea treatment compared to the positive control group, while there was no significant difference in the RNase activity estimated between the hydroxyurea treated group and the negative control group ($p < 0.05$), QYWS inhibited RNase significantly compared to the other experimental groups ($p < 0.05$), the RNase activity estimated in QYWS treatment was significantly lesser than the enzyme activity in negative control ($p < 0.05$). All the treatment samples significantly inhibited RNase activity compared to the positive control group ($p < 0.05$).

Activity of red cell carbonic anhydrase (RCCAase) was evaluated after 2-butoxyethanol induced hemolysis and disseminated thrombosis treatment by 1000 mg/kg BWT of QYWS, QYFS and QA, and hydroxyurea (Fig. 5). The activity of the RCCAase was significantly elevated by hydroxyurea and the quail egg components compared to the positive control group ($p < 0.05$). The estimated RCCAase activity after QYWS treatment had significant influence compared to the other treatment samples ($p < 0.05$). There was no significant difference in the influence of QA, QYFS and hydroxyurea on RCCAase activity ($p < 0.05$).

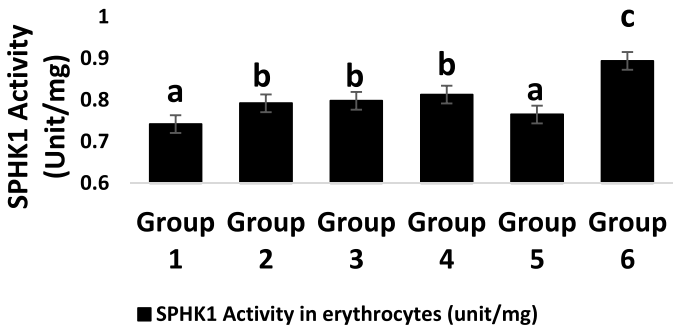

Fig. 2. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of Sphingosine kinase-1 in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

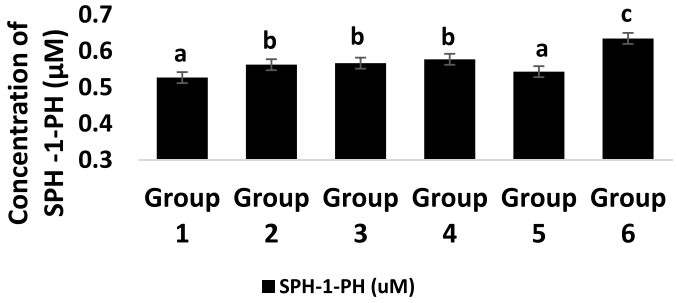

Fig. 3. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the concentration of Sphingosine-1 phosphate in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

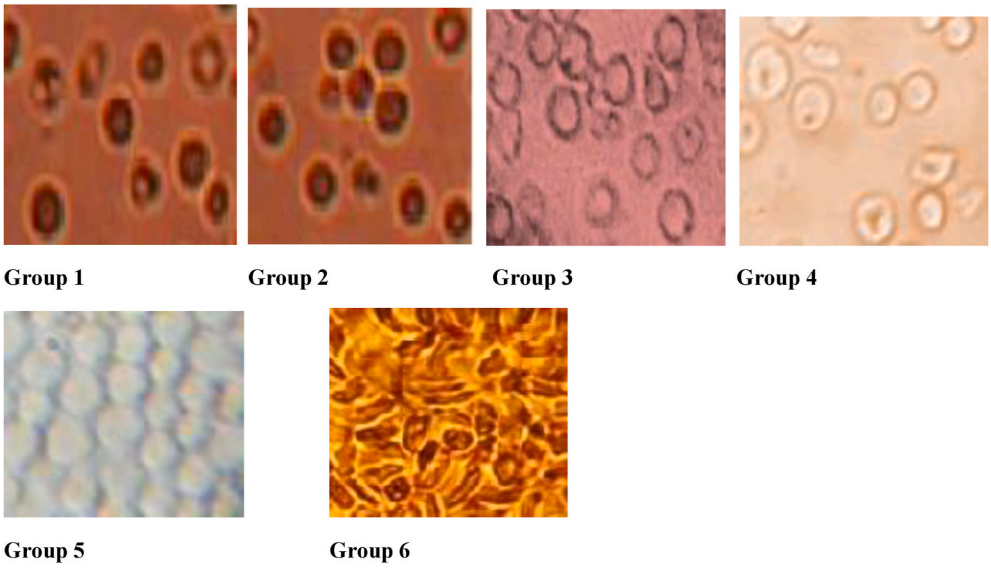

Fig. 1. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the morphology of the blood of female albino rats.

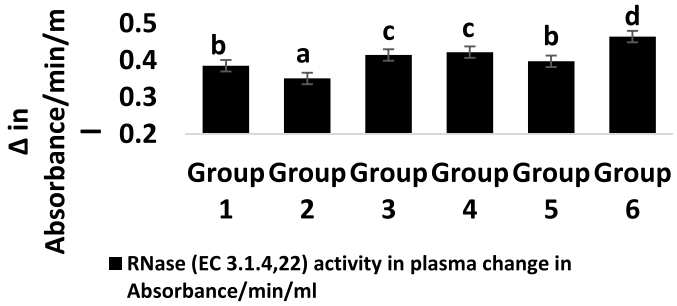

Fig. 4. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of RNase in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

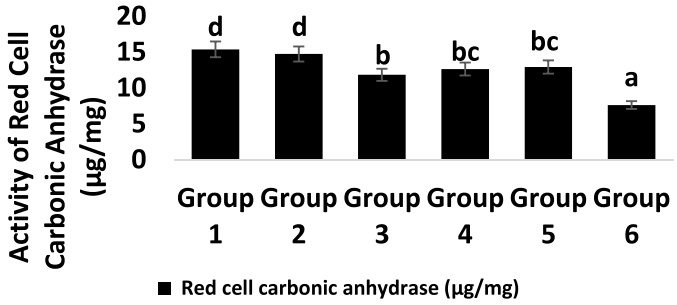

Fig. 5. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of red cell carbonic anhydrase in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

Figs. 6 and 7 revealed the effects of QYWS, QYFS and QA, and hydroxyurea on the concentrations of zinc ion in the plasma and urine respectively. 2-Butoxyethanol induction increased the rate of excretion of zinc which was evident from the significantly high urine zinc ion concentration and low zinc ion availability in the blood ($p < 0.05$). QYWS demonstrated the highest zinc ion retention in the blood, this was evident as it had the significantly least urine zinc ion concentration ($p < 0.05$) and highest plasma concentration that was not significantly different from the negative control group but significantly different compared to the influence of the other treatments ($p < 0.05$). QYFS, QA and hydroxyurea demonstrated zinc retention effects thus reversing the effects of 2-butoxyethanol in the positive control group ($p < 0.05$).

The influence of 1000 mg/kg BWT of QYWS, QYFS and QA as well as hydroxyurea on the activity of lactate dehydrogenase (LDH) was

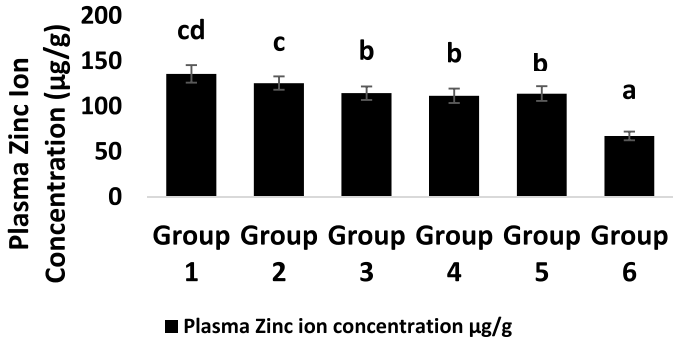

Fig. 6. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the concentration of plasma zinc in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

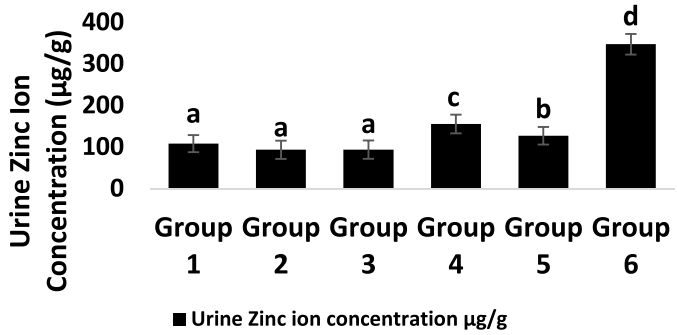

Fig. 7. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the concentration of urine zinc in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

investigated in the management of 2-butoxyethanol induced hemolysis and disseminated thrombosis in Fig. 8. The activity of LDH was significantly reduced by hydroxyurea compared to the other groups ($p < 0.05$). The quail egg components-QYWS, QWFS and QA had no significant difference ($p < 0.05$). All the experimental samples inhibited lactate dehydrogenase activity compared to the positive control group ($p < 0.05$).

The influence of 1000 mg/kg BWT of QYWS, QYFS and QA as well as hydroxyurea on the activity of glutathione peroxidase (GPx) was investigated in the management of 2-butoxyethanol induced hemolysis and disseminated thrombosis in Fig. 9. The activity of the GPx was significantly increased by treatments with hydroxyurea and the quail egg extracts-QYWS, QYFS and QA thus reversing the depression of immunological status against free radical progression initiated by 2-butoxyethanol in the positive control group ($p < 0.05$).

The influence 2-butoxyethanol induction on plasma selenium concentration and the ability of QYWS, QYFS and QA, and hydroxyurea to contend with the assault was revealed in Fig. 10. The concentration of selenium was significantly increased by QYWS, QYFS and QA, and hydroxyurea compared to the positive control group ($p < 0.05$). QYWS treatment increase selenium concentration significantly than the experimental samples ($p < 0.05$).

The effects of 2-butoxyethanol induced hemolysis and disseminated thrombosis on caspase-3 and the influence of the treatments with QYWS, QYFS and QA, and hydroxyurea (Fig. 11) was investigated. The caspase-3 activity was significantly reduced by QYWS, QYFS, QA and hydroxyurea, indicating a significant inhibition of the caspase-3 activity which was evident when compared to the positive control group ($p < 0.05$).

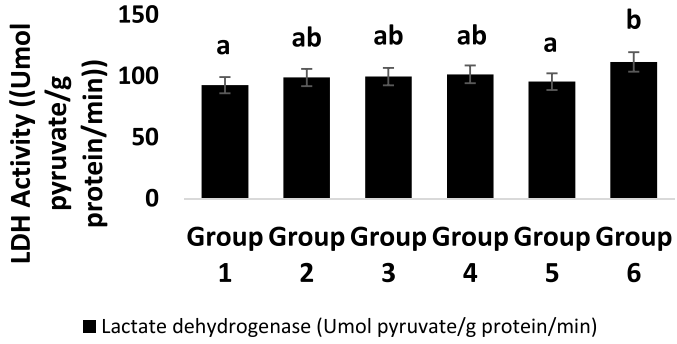

Fig. 8. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of lactate dehydrogenase (LDH) in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

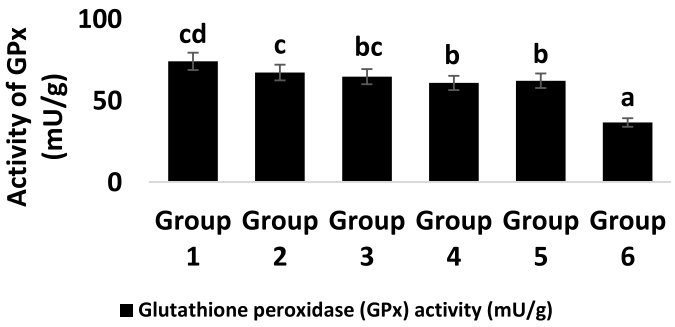

Fig. 9. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of GPx in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

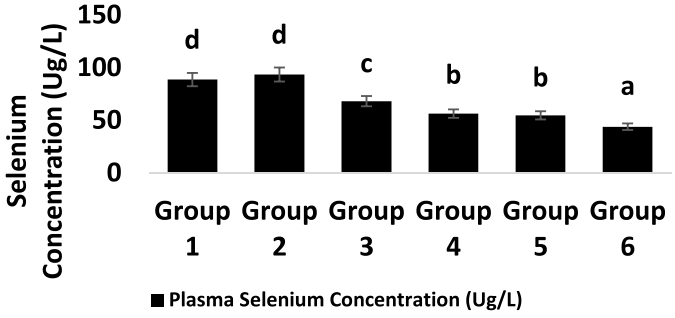

Fig. 10. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the concentration of selenium in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

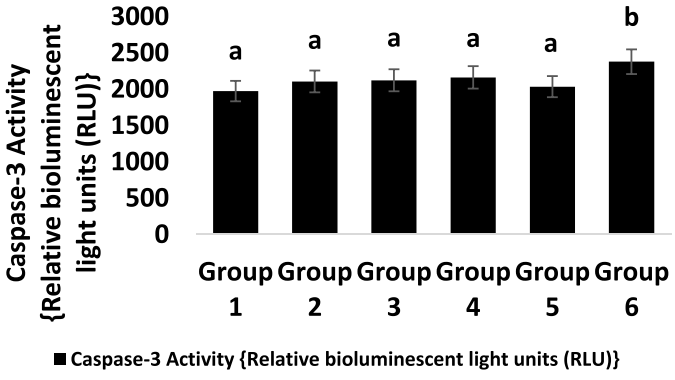

Fig. 11. Influence of Quail egg yolk water and fat soluble constituents, and albumen components on the activity of caspase-3 in hemolytic in blood of female albino rats. Results were presented as mean ± standard deviation where n = 5. Values with different superscript are significantly different ($p < 0.05$).

3.4. Evaluation of haematological parameters

The haematological evaluations revealed modulation of the blood parameters by quail egg yolk and albumen components-QYWS, QYFS and QA in Table 2. QYWS presented the best effects compared to hydroxyurea, QYFS and QA. The quail egg treatments ameliorated the 2-butoxyethanol-induced hemolysis, oxidative stress and disseminated thrombosis in female albino rats.

3.5. In vitro anti-inflammatory activities of quail egg yolk and albumen components

Percentage inhibition of inflammation by quail egg yolk fat-soluble (QYFS) and water-soluble (QYWS), and albumen (QA) was evaluated and revealed in Figs. 12–18. The anti-inflammatory effects of evaluated were inhibitions of albumen denaturation (Fig. 12), anti-proteinase activity (Fig. 13), heat-induced hemolysis (Fig. 14), hypotonicity-induced hemolysis (Fig. 15), lipoxygenase (Fig. 16), cyclooxygenase (Fig. 17) and β-glucuroidase (Fig. 18). The inhibition activities increased with the concentration increase. The standard anti-inflammation inhibitors used for each of the assays were-aspirin (albumin denaturation inhibition, anti-proteinase and inhibition of heat-induced hemolysis), diclofenac sodium (inhibition of hypotonicity-induced hemolysis), indomethacin (anti-lipoxygenase), ibuprofen (anti-cyclooxygenase) and silymarin (anti-β-glucuroidase). The components performed in similar order in all the investigations QYWS > QYFS > QA at major concentration (μg/ml), except in heat-induced hemolysis and lipoxygenase inhibition which revealed an activity trend of QYWS > QA > QYFS.

3.6. Quantification of bioactive components in quail egg yolk and albumen

The chromatography analysis of the quail egg yolk lipid extract as shown in Fig. 19 resulted in eleven peaks as obtained on the chromatogram of the fat-soluble and water-soluble yolk components. These peaks indicated the presence of eleven compounds in the extract of the quail egg yolk. The extract contained the fat soluble vitamins: vitamin A, D, E, and K. Other vitamins (water-soluble vitamins) presents are vitamin B3, B6, C, B1, B2, B9 and B5. The qualitative and quantitative evaluations of total vitamins in the sample revealed the following vitamins are present in milligram per 100 g of sample: vitamin B3: 0.04450; vitamin B6: 0.3790; vitamin C: 0.00004854; vitamin A: 0.4668; vitamin B1: 0.1762; vitamin B2: 0.5239; vitamin D: 0.005221; vitamin E: 2.634; Vitamin B9: 0.00004911; vitamin K: 0.0007144; vitamin B5: 2.873.

Fig. 20 showed the quality and amount of amino acids present in the quail egg albumen. Aspartic acid was the most predominant non-essential amino acid (NEAA) in the albumen with an amount of 1543.3 mg/100 g, followed by alanine (773.19 mg/100 g) and serine (538.53 mg/100 g). Leucine (1029.5 mg/100 g) was the most predominant essential amino acid (EAA) in present the albumen. Other amino acids such as valine (839.65 mg/100 g), threonine (793.88 mg/100 g) and lysine (794.02 mg/100 g) were discovered.

The quantification of the mineral elements present in the albumen and yolk was presented in Table 3. Manganese was not detected in the yolk and albumen, while phosphorus concentration in the yolk was 1361.2 ppm, the element was not detected in the albumen. Other elements such as selenium and copper were respectively 1932 ppm and 2 ppm in the yolk, but they were not detected in the albumen. The albumen had higher amounts of potassium, sodium, calcium, magnesium and nitrogen than the yolk sample. The yolk sample had predominant amounts of iron and zinc than the albumen samples in ppm.

4. Discussion

This study agreed with previous studies on the acute hemolysis, disseminated thrombosis and tissue infarction caused by 4-day administration of 250 mg/kg BWT of 2-butoxyethanol in female rats (Ezov et al., 2002; Lewis et al., 2005). The clinical features which indicated diagnosis of the effects of 2-BE were confirmed as reported in Ezov et al. (2002) and Lewis et al. (2005).

In this study, the morphological examination of the RBCs revealed that 2-BE caused a pathological loss of conformation of the RBCs, through RBCs swelling causing conformational changes which preceded hemolysis development. This sequence had been previously suggested and demonstrated in previous studies, in which the stomatocytes, or

Table 2
Effects of Quail egg yolk and albumen components on the blood parameters in female albino rats induced with 2-butoxyethanol hemolysis.

	Lymphocyte	Neutrophil	Monocyte	Eosinophil	PCV	RBC	WBC	HB	MCV	MCH
Group 1	27.17 ± 1.406	42.08 ± 3.058	8.35 ± 0.616	4.73 ± 0.209	42.48 ± 3.031	1840.2 ± 31.3	**565.83 ± 40.4**	**13.59 ± 0.970**	**56.74 ± 4.003**	**17.92 ± 1.279**
Group 2	24.74 ± 1.797	37.11 ± 2.292	8.87 ± 0.682	4.48 ± 0.304	39.39 ± 2.328	1603.2 ± 25.1	**481.46 ± 30.8**	**12.36 ± 0.74**	**51.57 ± 3.052**	**15.69 ± 0.976**
Group 3	20.91 ± 1.924	32.37 ± 2.316	9.85 ± 0.631	4.38 ± 0.328	32.68 ± 2.325	1415.2 ± 34.1	**435.14 ± 31.06**	**10.45 ± 0.746**	**43.22 ± 3.078**	**13.86 ± 0.984**
Group 4	22.33 ± 1.546	34.53 ± 2.47	9.22 ± 0.756	4.43 ± 0.317	34.97 ± 2.923	1512.1 ± 22.2	**464.9 ± 33.19**	**11.17 ± 0.795**	**46.77 ± 3.289**	**14.30 ± 1.05**
Group 5	22.28 ± 1.63	35.36 ± 2.547	8.90 ± 0.357	4.19 ± 0.299	35.61 ± 2.574	1545.7 ± 11.3	**475.2 ± 33.96**	**11.41 ± 0.812**	**47.99 ± 3.362**	**15.57 ± 1.075**
Group 6	13.56 ± 0.968	20.84 ± 1.482	10.40 ± 0.729	4.98 ± 0.504	21.02 ± 1.017	911.1 ± 65.1	**280.1 ± 20.0**	**6.73 ± 0.485**	**27.73 ± 1.982**	**8.75 ± 0.633**

	MCHC	Basophile	ESR
Group 1	39.16 ± 2.796	0	0.5 ± 0.037
Group 2	32.65 ± 2.324	1 ± 0.074	0.5 ± 0.037
Group 3	30.20 ± 2.506	1 ± 0.074	0.5 ± 0.037
Group 4	32.84 ± 2.279	1 ± 0.074	0.5 ± 0.037
Group 5	32.98 ± 2.349	1 ± 0.074	0.5 ± 0.037
Group 6	19.92 ± 1.384	1 ± 0.074	0.5 ± 0.037

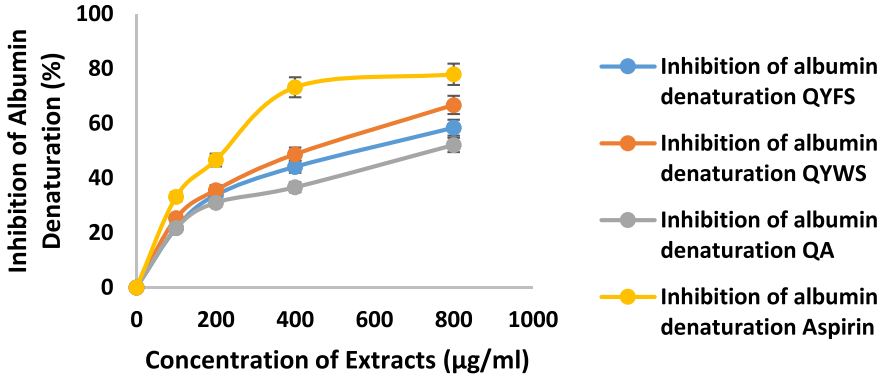

Fig. 12. Albumin denaturation inhibition activities of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

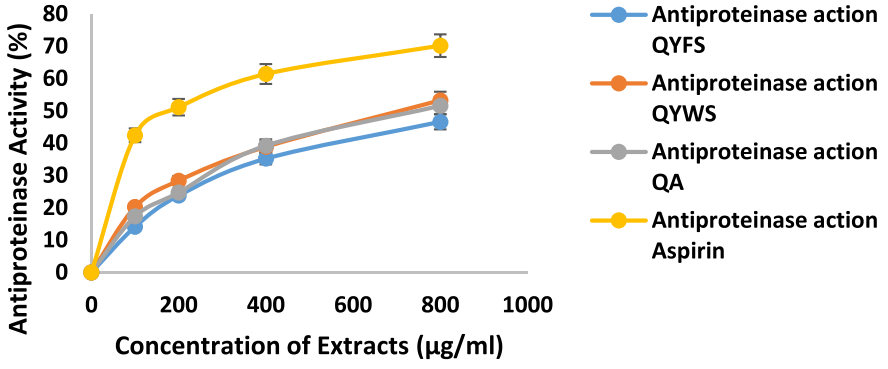

Fig. 13. Antiproteinase activities of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

cupped-shaped RBC, were formed soon after exposure to 2-BE, followed by the occurrence of spherocytosis and finally lysis (Ghanayem and Sullivan, 1993; Udden, 2000; Koshkaryev et al., 2003). Thombocytosis, coupled with the tendency of the platelets to clump to each other, and rouleaux formation, occurs 2–3 days following 2-BE exposure (Koshkaryev et al., 2003). However the knowledge on the cause of the conformational change is sparse, potentially, it could involve alteration of factors such as decrease in pH due to metabolic acidosis, circulation of the spleen, and exposure to shear stress in the circulation (Ezov et al., 2002; Koshkaryev et al., 2003). The quail egg components caused recovery of the RBCs structure from the assumed cupped or biconcaved shape. There were sparse similarity to this kind of design. Majority of experimental designs were confirmation of the hemolytic and disseminated thrombotic effects of 2-BE (Ezov et al., 2002; Koshkaryev et al., 2003; Lewis et al., 2005). However, studies had investigated the influence of some folkloric samples on the conformation of the RBCs, some of the samples investigated in a particular study were *C. cajan* seed, *C. cajan* leaf, *Z. zanthoxyloides* leaf, and *C. papaya* leaf (Nurain et al., 2017), others were *Dicliptera colorata*, *Euphorbia hirta* L. and *Sorghum bicolor* L. (Mpiana et al., 2013). All these plants demonstrated

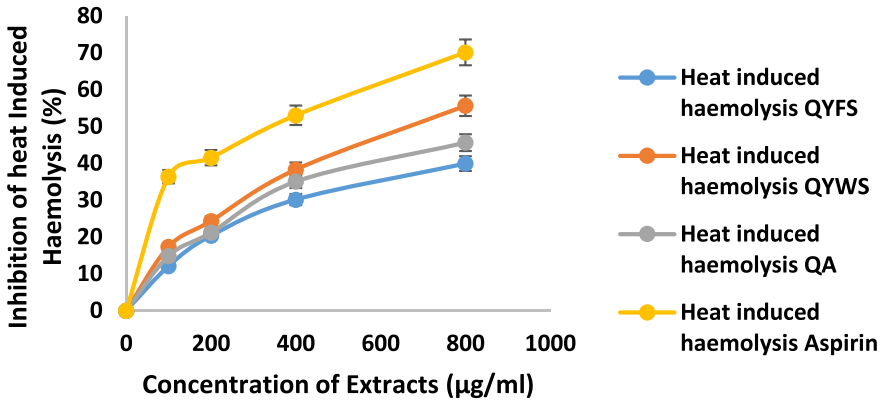

Fig. 14. Inhibition of heat-induced haemolysis of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

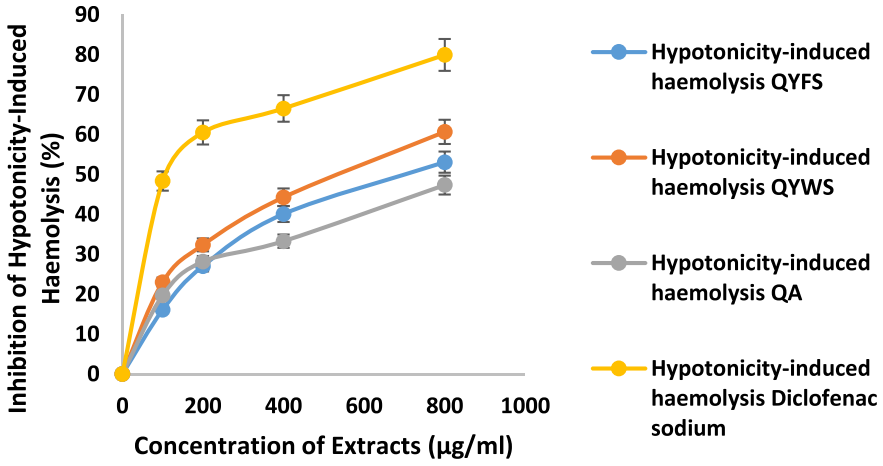

Fig. 15. Inhibition of Hypotonicity-Induced haemolysis of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

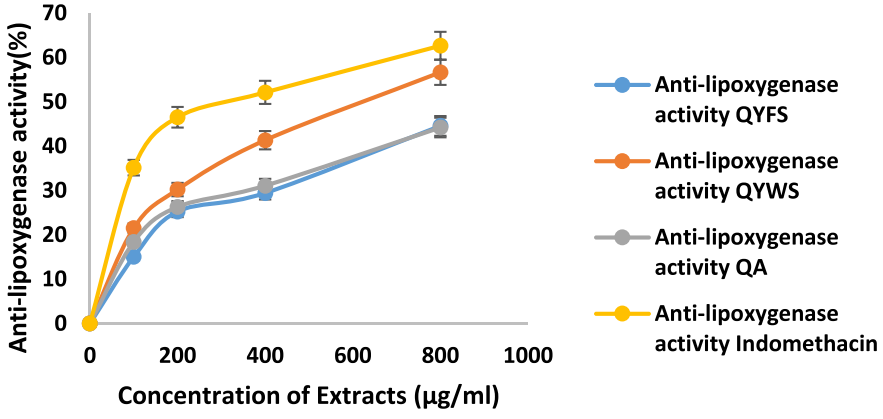

Fig. 16. Anti-lipoxygenase activity of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

anti-hemolytic effects of different degrees, similar to the influence of quail egg components.

Non-biased high-throughput metabolomics profiling, has revealed sphingosine-1-phosphate which is a bioactive lipid molecule, highly enriched in erythrocytes and plasma of mice and humans with sickle cell disease (Zhang et al., 2014). Sphingosine-1-phosphate is important signal transduction as a molecule, which regulates diverse biochemical processes, which include inflammation, angiogenesis, endothelial injury, and thrombosis (Zhang et al., 2014) via activated cell surface sphingosine-1-phosphate receptors and/or by interaction with key regulatory proteins within cells (Spiegel and Milstien, 2003). Sphingosine-1-phosphate is generated intracellularly by sphingosine kinase 1 and 2. Erythrocytes are unique among cells because they lack sphingosine-1-phosphate degrading enzymes (Zhang et al., 2014). This study established that 2-BE could activate sphingosine kinase-1 which in turn catalyzed the metabolism of sphingosine-1 phosphate, this could be a biochemical channel for inflammation, angiogenesis, thrombosis and hemolysis which had been reported as the characteristic features of 2-BE

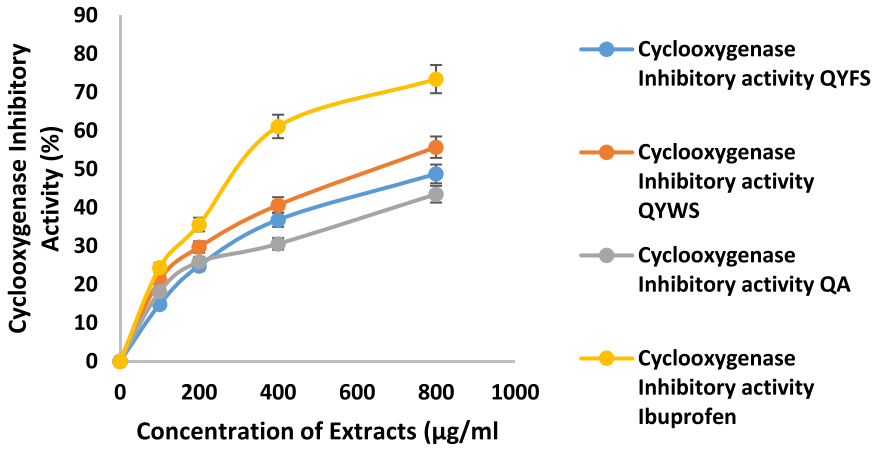

Fig. 17. Cyclooxygenase inhibitory activity of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

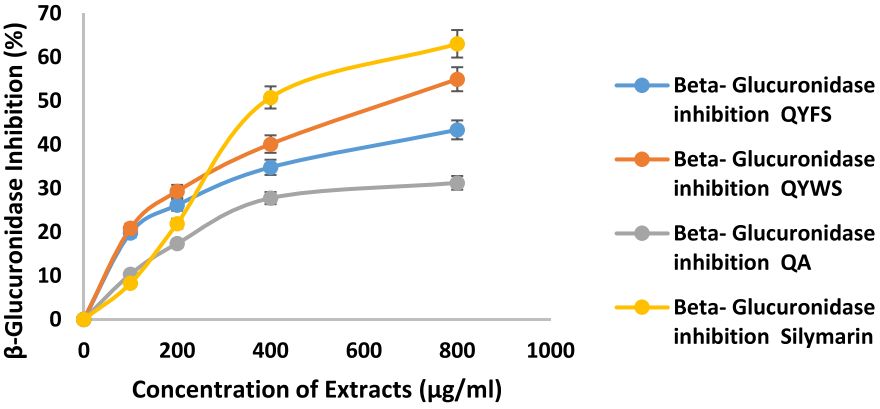

Fig. 18. β-glucuronidase inhibition of Quail egg yolk and albumen components. Results were presented as mean ± standard deviation where n = 3 ($p \leq 0.05$).

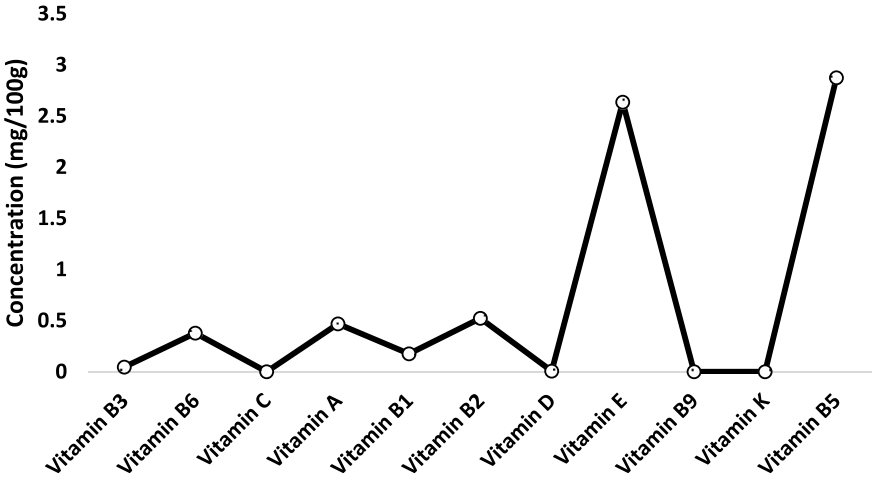

Fig. 19. Vitamins composition in QYFS and QYWS.

induced depranocytosis. Quail egg components demonstrated an inhibiting influence on sphingosine kinase-1, impeding the metabolism of the sphingosine-1-phosphate, which is possible therapeutic model in the management of depranocytosis. Using animal model, the study had confirmed that sphingosine kinase-1 and sphingosine-1-phosphate are significantly elevated in patients with SCD. The finding had shown that the quail egg components contains compounds which could be potent inhibitors of sphingosine kinase-1. Moreover, it was revealed that inhibition of sphingosine kinase-1 activity by these nutritional inhibitors and hydroxyurea significantly reduced 2BE-induced sickling in Wistar rats RBCs. These study provided strong evidence that quail egg attenuation of 2-BE-induction regulated sphingosine kinase-1 activity resulting in decreased sphingosine-1-phosphate production and directly managing the sickling of the RBCs.

Studies supported by clinical and biochemical data had shown that sickle cell disease patients are deficient of plasma zinc. Zinc has been

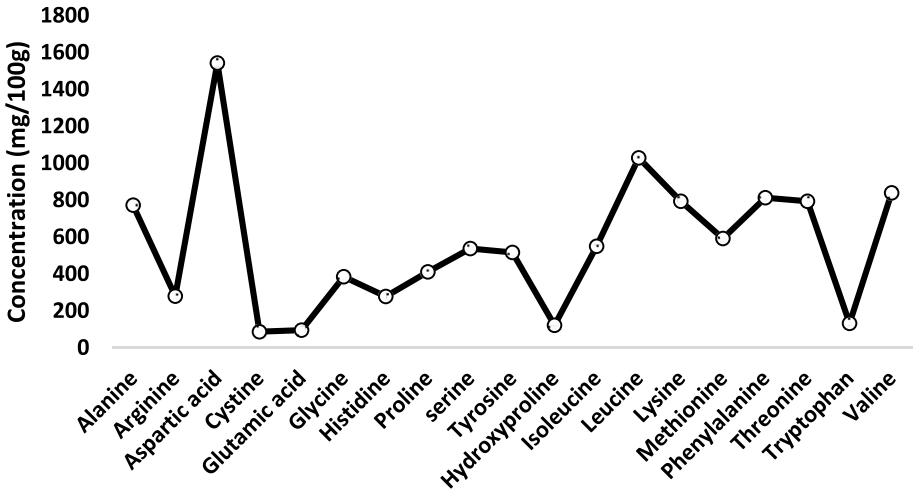

Fig. 20. Amino acids composition in QA.

Table 3
Mineral elements composition in quail egg yolk and albumen.

	K	Na	Ca	Mn	Fe	Cu	Zn	P	Mg	N	Se
YOLK	763	499	53150	ND	69	2	66	1361.2	18271	49	1932
ALBUMEN	1062	2966	56700	ND	14	ND	22	ND	32981	69719	ND

implicated in growth retardation, fibroplastic proliferation and collagen synthesis, deoxyribonucleic acid (DNA), DNA-dependent RNA polymerase and thymidine kinase activities (Prasad et al., 1975). As long as zinc is an essential component of the RBCs, it is possible that long-continued hemolysis in patients with sickle cell disease might lead to a zinc-deficient state, which could account for some of the clinical manifestations of zinc deficiency (Prasad et al., 1975). This study had provided useful link between 2-BE induction and increased zinc loss and excretion as a result of hemolysis, Quail egg components reversed hemolytic impairments caused by 2-BE, thus retaining zinc which had been reported as a constituent of RBCs and this was evident from the reduced urinary zinc concentration after the treatments with quail egg components. Activity of RNase was reported to be inhibited by zinc (Prasad and Oberleas, 1973). Thus an increase in RNase activity in plasma of sickle cell disease patients may be indicative of zinc deficiency (Prasad et al., 1975). This study had further revealed the link between 2-BE and enzymatic dysregulations that are characteristic features in sickle cell disease, this study showed that 2-BE assault activated the RNase, which was complementary of its effects on plasma zinc. Quail egg components demonstrated inhibitory influence over the RNase which could on one part be as a result of plasma zinc elevating potential of the treatments. Another enzyme dependent on zinc availability is red cell carbonic anhydrase, which is activated in the presence of zinc, and inhibited in sickle cell disease condition (Prasad, 2002), however the enzyme activity was inhibited by 2-BE assault but the enzyme activation was demonstrated by quail egg treatments, it is suggestive that the influence on the carbonic anhydrase is the quail egg components zinc elevating potential. The inorganic minerals determination on the albumen and yolk showed the presence of zinc in the components, however, yolk sample had the highest amount of zinc, which had reflected in its influence on plasma zinc concentration and the activities of RNase and carbonic anhydrase.

Accelerated oxidative damage is a hallmark in sickle cell disease. Patients with sickle cell disease have manifested reduced levels of selenium, glutathione, vitamins A, C, riboflavin (B2) (Hamdy et al., 2015), pyridine (B6), folic acid (B9), cobalamin (B12), D and E. Besides the reported anti-sickling activities of some of the aforementioned, they also demonstrate significant attenuation of oxidative stress through the scavenging of free radicals that are overwhelming the antioxidant status in the animal or human system. 2-BE induction initiated the progression of free radicals and oxidative stress, which was evident from the reduced activity of glutathione peroxidase. In this study, 2-BE caused a significant reduction in the concentration of selenium, the relationship between selenium and sickle cell disease had been explained as attenuation of chronic oxidative stress (Hamdy et al., 2015). Hamdy et al., 2015 suggested that selenium concentration in the blood reflects recent selenium intake and not the long term intake. This justifies the influence of the quail egg yolk extracts-QYFS and QYWS, which from the inorganic mineral elements composition showed the presence of selenium in the yolk. Although the study also revealed selenium elevating potentials of albumen extract and hydroxyurea, there was no evidence of selenium presence in the albumen, and the mechanism of elevation of selenium by hydroxyurea was not established from literatures.

Angiogenesis has been demonstrated in sickle cell disease (French et al., 2006). Studies have shown the link between pathways of angiogenesis and the implications of sphingosine kinase-1, the key factors of interest in similar studies are proinflammatory cytokines-TNF-α, IL-1β and IL-6 as well as cysteine-aspartyl specific proteases (Caspase) (French et al., 2006) that play key role in apoptosis and cytokine activation (Liadis et al., 2005), coordinated majorly by caspase-3.2-BE demonstrated apoptotic effect through the activation of caspase-3. Quail egg components demonstrated anti-apoptotic influence by the inhibition of caspase-3 activities.

In conclusion, this study had contributed to the available knowledge on the mechanism of experimental model of 2-BE in female Wistar rats, with emphasis on the activation of red cell carbonic anhydrase, lactate dehydrogenase, caspase-3, RNase and sphingosine kinase-1 which are key enzymes associated to hemolysis and thrombosis in depranocytotic subjects. Quail egg contains selenium, zinc, iron, magnesium as well as calcium which are essential in the inhibition of these deleterious enzymes and necessary for elevating antioxidant potential in depranocytotic subjects. The morphologically cup-shaped RBCs as a result of 20-BE was reversed by quail egg components. Hydroxyurea demonstrated antihemolytic effects against 2-BE, thus justified the use as standard antisickling drug, but the use of quail egg is suggested to annul the possibility of hepatotoxicity that had been reported by hydroxyurea.

Declaration of competing interest

There is no conflict of interest among authors.

Acknowledgements

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