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Aqueous extract of *Carica papaya* Linn. roots potentially attenuates arsenic induced biochemical and genotoxic effects in Wistar rats

Oluwafemi Adeleke Ojo [a, *], Adebola Busola Ojo [b], Olayinka Awoyinka [c], Basiru Olaitan Ajiboye [a], Babatunji Emmanuel Oyinloye [a], Olukemi Adetutu Osukoya [a], Israel Idowu Olayide [a], Adejoke Ibitayo [d]

[a] *Phytomedicine, Biochemical Toxicology and Diabetes Research Laboratories, Department of Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*
[b] *Department of Medical Biochemistry, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*
[c] *Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Ekiti State University, Ado-Ekiti, Nigeria*
[d] *Department of Biological Sciences, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria*

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ABSTRACT

In Africa, the fruit, leaf, seed and roots of *Carica papaya* Linn. are generally used to treat a variety of diseases such as malaria, cancer, and cardiovascular diseases. In this study, we evaluated the protective potentials of aqueous extract of *C. papaya* roots on arsenic-induced biochemical and genotoxic effects in Wistar rats. Rats were induced intraperitoneal with sodium arsenate (dissolved in distilled water at 3 mg/kg body weight) for 21 days and the animals were administered simultaneously with 200 mg/kg body weight vitamin C, 100 and 150 mg/kg body weight of the *C. papaya* Linn. root aqueous extract once daily for three weeks. Results obtained reveals that activities of plasma 8-OHdG, serum lipids concentration, atherogenic index (AI), coronary artery index (CRI), aspartate transaminase, alanine transaminase, alkaline phosphatase, total bilirubin levels were elevated significantly ($p < 0.05$) and catalase, glutathione peroxidase, superoxide dismutase, plasma hematological profile were progressively reduced ($p < 0.05$) in arsenic-alone exposed rats. Significant increase in the quantity of chromosomal aberrations (CA), micronuclei (MN) frequency, oxidative damages in the bone marrow cells from arsenic alone rats was observed. Though, mitotic index scores in these cells were progressively reduced ($p < 0.05$). In animals administered with aqueous extract of *C. papaya* roots and vitamin C, the altered parameters were significantly recovered towards the levels observed in normal control rats. These results suggest that aqueous *C. papaya* roots preparations might have therapeutic potential as a supplement that can be applied in arsenic poisoning.

1. Introduction

Arsenic is spread broadly in the natural world, existing as a constituent of organic or inorganic molecules. Arsenic inorganic compounds include arsenite, which is considered as the most poisonous form, and arsenate, whereby organic arsenic-containing molecules are usually less poisonous.[1] Due to the broad spread of arsenic in nature, humans get in contact with this heavy metal very often. The usual way to get in contact with inorganic arsenic is for example via water. Prolonged contact to arsenic leads to a broad range of damaging effects. This heavy metal is classified as Class I human cancer-causing agent.[2] Acute intake of arsenic induces injury to tissues such as kidney, liver, intestine, and brain.[3–6]

Epidemiological research has reported a robust connection between prolonged exposure to arsenic and deleterious health effects, such as cardiac diseases, neurological disorders and cancer[7–10]. Sub-chronic contact to arsenic via water modifies the expression of cancer genes in tissues.[11] Arsenic exerts its damaging effects via increase of oxidative stress. Moreover, free radicals were detected in liver and kidney cells treated with arsenite.[12,13] Boulikas,[14] has

* Corresponding author. Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Ado-Ekiti, Nigeria.
E-mail address: oluwafemiadeleke08@gmail.com (O.A. Ojo).
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List of abbreviation

As	Arsenic
AST	aspartate transaminase
CA	chromosomal aberrations
CAT	catalase
C. papaya	*Carica papaya* Linn.
GSH	glutathione reduced
GPx	glutathione peroxidase
HDL	high density lipoprotein
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MN	micronuclei
MPCE	multinucleated polychromatic erythrocytes
NCE	normochromatic erythrocyte
PCE	polychromatic-erythrocytes
8-OHdG	8-hydroxydeoxyguanosine
Hb	hemoglobin

shown that ROS are implicated in oxidative damage inflicted from arsenic to macromolecules in cells, leading to apoptosis.

Though, arsenic cannot cause mutation in cell culture, it was absolutely shown to enhance the toxicity, mutagenicity, and clastogenicity of UV-radiation, and alkylating and deoxyribonucleic acid crosslinking agents in rodents and human cells.[15,16] Lee et al.[17] mentioned that the genotoxic research of arsenic yielded no effect for sequence mutations, however, encouraging results for chromosomal anomalies. Analysis for genotoxicity designated that arsenic compounds restrain deoxyribonucleic acid repair, as well as incite chromosomal anomalies and micronuclei formation in rat cultured cells,[18] and in cells of exposed humans.[19] 8-OHdG is formed from deoxyguanosine in DNA by hydroxyl free radicals. Because of its stability, 8-OHdG is known as one of the most reliable markers of oxidative DNA damage.[20]

Besides, *in vitro* cell transformation tests, proved to be a good approach to obtain mechanistic data on the neoplastic potential of arsenic exposure. Many synthetic antidotes, such as British anti-lewisite and dimercapto propane 1-sulphonate were employed in controlling arsenic damaging potential. Synthetic antidotes chelate completely with sulfhydryl groups resulting in the removal of arsenic. Though, standard metal-chelating agents have undesired effects such as nausea, vomiting, and fever,[21] the possibility of exploring natural products to prevent the toxic effects of arsenic, is of high interest. Tests for genotoxicity have indicated that arsenic compounds inhibit DNA repair, and induce chromosomal aberrations, sister-chromatid exchanges, and micronuclei formation in both human and rodent cells in culture[18,22,23] and in cells of exposed humans.[19]

Plants have proven to be a good source for the development of therapeutic preparations.[24] *Carica papaya* Linn. belongs to *Caricaceae* family. Papaya is a succulent plant that possesses self-sustaining twigs.[25] It's an outsized perennial herb with a swift rate of growth. This species is typically not a long-living, however, it yields fruits for over twenty years.[26] The papaya has a rather complex means of reproduction which can be classified as male, hermaphrodite, or female.[27] Hermaphrodite trees are standard for commercial use, yielding pear-formed fruits. Self-pollination is typical for the plants,[28] originally derived from the southern part of Mexico. It has become established in many tropical and subtropical countries.[29] In general, *C. papaya* fruits are used extensively and valued as food. In Nigeria, pawpaw is one among the prevalent, low cost and economically valuable fruit trees, valued for its dietary properties.[30] The top three countries that produce *C. papaya* plants worldwide are; Brazil (25%), Federal Republic of Nigeria (15%), and India (12%).[31] Maisarah et al.[32] reported the anti-oxidative properties of various components of *C. papaya* except for root. Hence, the goal of this study is to check the geno-protective potentials of aqueous extract of *C. papaya* roots against arsenic-induced genotoxic effects.

2. Materials and methods

2.1. Plant material

Roots of *C. papaya* were freshly purchased in May, 2016 from Ijadu farm, Ado-Ekiti, Nigeria. The plant was authenticated and documented by a senior taxonomist at the herbarium unit of the Department of Biological Science, Afe Babalola University, Ado-Ekiti, Nigeria and an herbarium number ABUAD/2016/040 was documented. Roots sample was instantly washed and air-dried for 2 weeks. Dried roots were minced to powder and then kept in a sealed vessel for further investigation.

2.2. Preparation of aqueous extract of C. papaya root

The aqueous extract of the powder *C. papaya* root was prepared using the modified method of.[33] Fifty grams of the fine-grained sample were extracted with water (via maceration) for 48 h. The percentage extract yield calculated as follows:

$$\%\ \text{yield} = \frac{\text{Weight of the dry extract}}{\text{Weight of fine} - \text{grained roots}} \times 100\%$$

2.3. Arsenic induction

Arsenic in the form of inorganic arsenic (sodium arsenite, NaAsO2, Mol weight 129.9) obtained from Sigma was used for the present study. The dose 3.0 mg/kg body weight was selected for the present experiment (one-tenth of lethal dose). Sodium arsenite in distilled water was administered intraperitoneal immediately.

2.4. Experimental animals

Male Wistar rats with initial mean body weight (155.25 ± 11.52 g) were employed in this work. The animals used were purchased from Animal house, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria. The animals were housed in big metabolic cages and were feed food and given water *ad libitum*. The animal chamber was ventilated and with a (6 am–6 pm) light/dark cycle, throughout the period of the experiment. Animals were maintained in line with the directions and principles of the Experimental Animal Research Ethics Committee of Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria (Ethical approval number: ABUAD/ACA/121).

2.5. Animal grouping

Animals were randomly assigned into five groups, as follows: Group I rats received distilled water vehicles alone (normal control), Group II rats received in arsenic as sodium arsenite (at 3 mg/kg body weight) intraperitoneal once daily for 21 days, Group III animals received arsenic as sodium arsenite (at 3 mg/kg body weight) intraperitoneal + water-soluble vitamin C (200 mg/kg

body weight dissolved in water) by oral gavage once every day, Group IV animals received arsenic as sodium arsenate (at 3 mg/kg body weight) intraperitoneal + aqueous extract of *C. papaya* roots (100 mg/kg body weight) orally once every day, and Group V animals received arsenic as sodium arsenate (at 3 mg/kg body weight) intraperitoneal + aqueous extract of *C. papaya* roots (150 mg/kg body weight) orally once daily for 21 days.

2.6. Isolation of blood and organs

Animals were euthanized by inhalation of anesthetic and organ and blood samples were taken on the twenty-second (22) day. Blood from every animal was collected via heart puncture and directly frozen at -30 °C till additional processing. Blood was allow put in a clean tube and allow to clot, centrifuged at 3000 rpm for 10 min and blood serum was removed and preserved at -30 °C for additional analysis. Liver from every animal was removed, washed, weighed and preserved till later use. A bone marrow cells sample from every animal was taken and used to study chromosomal aberrations.

2.7. Determination of in vitro antioxidant activity

2.7.1. Estimation of total phenol content

The total phenol content of *C. papaya* roots aqueous extract was assessed (as gallic acid equivalent) by the described procedure by.[34] In short, 200 μL extract dissolved in 10 % DMSO (240 μg mL^{-1}) was incubated with one mL of Folin Ciocalteau chemical agent (diluted 10 times) and 800 μL of 0.7 mol L^{-1} Na_2CO_3 for 30 min at ambient temperature. Absorbance was read at 765 nm using spectrophotometer. All readings were repeated three times. Results expressed as mg GAE per hundred grams of dry aqueous extracts.

2.7.2. Determination of ferric reducing antioxidant property (FRAP) of C. papaya roots aqueous extract

The reducing property of the *C. papaya* roots aqueous extract was studied by assessing the power of the extract to reduce $FeCl_3$ solution as described by Pulido et al.[35] A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Solution was incubated for 20 min at 50 °C in a water bath and then 2.5 mL of 10% trichloroacetic acid was added. The sample was then centrifuged at 650 g for 10 min. After that, 5 mL of the supernatant was mixed with an equal water volume and one mL, 0.1% $FeCl_3$. The above-stated process was also applied to a standard ascorbic acid solution, and finally the absorbance was read at 700 nm. The reducing ability was calculated as percentage inhibition.

2.7.3. Determination of iron chelation ability

The Fe^{2+}chelating ability of aqueous *C. papaya* roots extract was determined by employing a changed procedure by Minotti and Aust[36] with a small adjustment by Puntel et al.[37] Newly made 500 μmol L^{-1} $FeSO_4$ (150 μL) was added to the solution containing 168 μL of 0.1 mol L^{-1} Tris-HCl (pH 7.4), together with 218 μL saline and the aqueous extract (1–5 mL). The solution was incubated for 5 min, with following addition of 13 μL of 0.25% (w/v) of 1, 10-phenanthroline. Absorbance was read at 510 nm Fe^{2+} chelating ability was determined and expressed as percentage inhibition.

2.7.4. NO free radical scavenging activity

Nitric oxide from sodium nitroprusside in solution at pH (7.0) reacts with oxygen to yield nitrite ions that are quantified by the Griess reaction. Nitric oxide scavengers compete with oxygen resulting in a diminished generation of nitric oxide gas.[38] The solution (3 mL) containing sodium nitroprusside (10 mM, in PBS) and also the extract in different dilutions (1, 2, 3, 4 and 5 mg/mL) was incubated for 150 min at 25 °C. At each thirty min interval, 0.5 mL of the sample was taken out and mixed with 0.5 ml of Griess reagent (1% sulphanilamide and 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). Absorbance was read at 546 nm. Analyses were repeated three times. The percentage inhibition of NO production was calculated.

2.7.5. Estimation of plasma 8-hydroxydeoxyguanosine (8-OHdG)

Blood plasma samples from rats from every group were diluted twofold and filtered (20 μm filters). After that, samples derived were used for 8-OHdG quantification. The estimation was done using the NWLSTM 8-OHdG ELISA assay kit. The proteins in every sample were also assessed, and results were calculated as 8-OHdG (ng/mg of protein).

2.7.6. Determination of serum lipid and other liver function related parameters

The serum lipid profile, total bilirubin, amino acid aminotransferases (AST and ALT), and alkaline phosphatase enzyme (ALP) were quantified using an Automatic Chemistry Analyzer (Brazil) with commercial assay kits from the same company. The liver antioxidant enzyme activities, catalase (CAT) were determined by adopting the method as described Sinha,[39] enzyme superoxide dismutase (SOD) was assessed based on Misra and Fridovich [40] and glutathione peroxidase (GPx) was analyzed by Hafeman et al.[41] based on the decomposition of H_2O_2, malonaldehyde (MDA) levels were determined via evaluating thiobarbituric acid-reactive substances according to Varshney and Kale.[42] LDL-cholesterol was computed via the equation shown below Friedewald et al.[43]:

LDL-cholesterol (mg/dl) = [TC- HDL- (TG/5)]

Where, TG/5 corresponds to the concentration of Very low density lipoprotein-cholesterol (VLDL-cholesterol), TG-triacylglycerol.

Atherogenic index (AI) was calculated as described by Liu et al.[44]
Atherogenic index (AI) = (TC-HDL-cholesterol)/HDL-cholesterol
Where, TC is total cholesterol, HDL is high density lipoprotein.
Coronary artery risk index (CRI) was determined as described by Boers et al.[45]:
Coronary artery risk index (CRI) = TC (mg/dl)/HDL-cholesterol (mg/dl).

2.8. Haematological analysis

Blood samples were collected from the animals through heart puncture. Hematological parameters for example haemoglobin (Hb), packed cell volume (PCV), white blood cells (WBC) count, WBC percentage composition were assessed via automated analyser. WBC count was analysed by haemocytometer protocol, whereas the smears were prepared by the Leishman procedure and evaluated by counting technique. Micro haematocrit technique was used to analysed the PCV, while cyanomethaemoglobin procedure was used to estimate the concentration of Hb.[46] Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were analysed by an automatic analyser.

2.9. Estimation of arsenic

Tissue and blood samples were processed in line with the procedure described by Ballentine and Burford.[47] 1 ml of concentrated nitric acid was mixed with 100 mg of tissues (or mL of blood), and afterward 1 mL of perchloric acid was added. Samples were then

incubated in a sand bath till the formation of yellow appearance. If the color remained still brown, additional nitric acid and perchloric acid was added and further incubation was done. The digest was filled up until a desired volume with distilled water. A little amount of the sample was used to evaluate arsenic through atomic absorption spectrophotometer. The amount of arsenic was calculated as μg/dL blood or μg/g tissue.

2.10. Cytogenic study

The micronucleus assay was performed to identify chromosomal changes according to the well-known procedure by Agarwal and Chauhan.[48] Bone marrow cells of every rat within the groups were placed into a separate glass slide and made uniform with several drops of fetal calf serum. Isolated cells were smeared, dried to be fixed in methyl alcohol, and visualized by staining with Geimsa in phosphate buffer pH 6.8. Polychromatic erythrocytes (PCE 1000 per animal) were examined for micronuclei, and variations in mitotic activity[49,50] was evaluated based on polychromatic to normochromatic erythrocytes ratio (PCE/NCE ratio).

2.11. Mitotic index estimation

Mitotic index was determined to define outline the degree of cell proliferation. Slides for the evaluation of chromosomal anomalies were similarly processed in analyzing mitotic index. Casually particular areas from the slides were examined to define the quantity of cells proliferation (metaphase stage) and also the total number of cells in the given area of the slide. As a minimum, 1000 cells were studied per determination.

2.12. Data analysis

Quantifications are displayed as mean ± SD (n = 5). Data were evaluated using one-way analysis of variance (ANOVA) via IBM software package (SPSS, Version 20.0) using Tukey's HSD multiple range *post hoc* test. Values expressed were regarded a significantly different when $p < 0.05$.

3. Results

3.1. Estimation of total phenol content

The yield of aqueous extract of *C. papaya* root was about 15.2%. The total phenolic content of aqueous extract of *C. papaya* root increases as the concentration (1–5 mg/mL) of extract increases as presented in Table 1. At 5 mg/mL, the extract of *C. papaya* has high phenol content of 79.92 ± 1.12 mg GAE/100 g.

3.2. Evaluation of ferric reducing antioxidant ability and nitric oxide scavenging ability

Aqueous extract of *C. papaya* root exhibits significantly ($p < 0.05$) higher reducing power compared to vitamin C as displayed in Fig. 1.

Table 1
Phenolic content of the *Carica papaya* root aqueous extract.

Concentration (mg/ml)	(mg GAE/100 g)
1	19.88 ± 1.09
2	29.80 ± 1.13
3	32.75 ± 1.11
4	54.85 ± 1.01
5	79.92 ± 1.12

* Results are expressed as mean ± SD.

The reducing potential of the extracts was increasing with the concentration increase (1–5 mg/mL), as shown in Fig. 1. The inhibitory effect of the extracts at the concentration of 1–5 mg/mL on NO free radical increased as the concentrations of *C. papaya* extracts (Fig. 2) increases. IC_{50} value for NO radical scavenging ability of the aqueous extracts of *C. papaya* root was 1.68 mg/mL. In this study, the *C. papaya* extracts at the concentrations used displayed substantial DPPH scavenging activity signifying its ability to perform as a radical scavenger.

3.3. Evaluation of iron chelating ability of C. papaya root aqueous extract

C. papaya root aqueous extract had higher chelating effect ($p < 0.05$) compared to vitamin C. IC_{50} values for the chelating effect of aqueous extracts of *C. papaya* root was 3.29 mg/mL. The chelating effect increased with increased concentration of *C. papaya* root (1–5 mg/mL) as shown in Fig. 3.

3.4. Evaluation of 8-OHdG level

The data for the oxidative stress marker of DNA in plasma are shown in Fig. 4. The 8-OHdG level was increased ($p < 0.05$) in arsenic alone exposed rats compared to untreated rats. However, treatment with aqueous extract of *C. papaya* root at 100 and 150 mg/kg body weight significantly reduced the level of 8-OHdG in plasma compared with arsenic exposed rats. Vitamin C treatment also significantly reduced the levels of 8-OHdG ($p < 0.05$) (Fig. 4). The effects were more pronounced in the arsenic + 150 mg/kg body weight *C. papaya* root aqueous extract compared to Vitamin C treated animals.

3.5. Evaluation of serum lipid profile, atherogenic and coronary risk indices

Serum lipid concentrations, as well as derived atherogenic index (AI) and coronary risk index (CRI) scores are displayed in Table 2. Elevation in serum concentrations of triacylglycerol, total cholesterol, and LDL-cholesterol levels along with AI and CRI scores with successive decrease in HDL-cholesterol, were witnessed in arsenic-only exposed rats comparable to control rats (Table 2). Administration of *C. papaya* root aqueous extract decreased ($p < 0.05$) the concentration of triacylglycerol, total cholesterol, LDL-cholesterol, atherogenic and coronary risk index compared to the arsenic only treated group. Significant increase in serum HDL-cholesterol level was also witnessed in the *C. papaya* root aqueous extract treated groups (Table 2).

3.6. Assessment of serum liver markers

Data about serum ALT, AST, ALP, and bilirubin are presented in Table 3. The levels of these serum factors were increased in the arsenic-only exposed group comparable to control group. On the other hand, treatment with *C. papaya* root aqueous extract significantly ($p < 0.05$) ameliorated these alterations at 100 mg/kg and 150 mg/kg body weight dose. The effects remained more noticeable in the arsenic + 150 mg/kg body weight of *C. papaya* root aqueous extract group compared to Vitamin C treated animals regarding most of the parameters.

3.7. Evaluation of haematological parameters

The data for haemoglobin (Hb) levels, red blood cells (RBCs), packed cell volume (PCV), white blood cells (WBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular

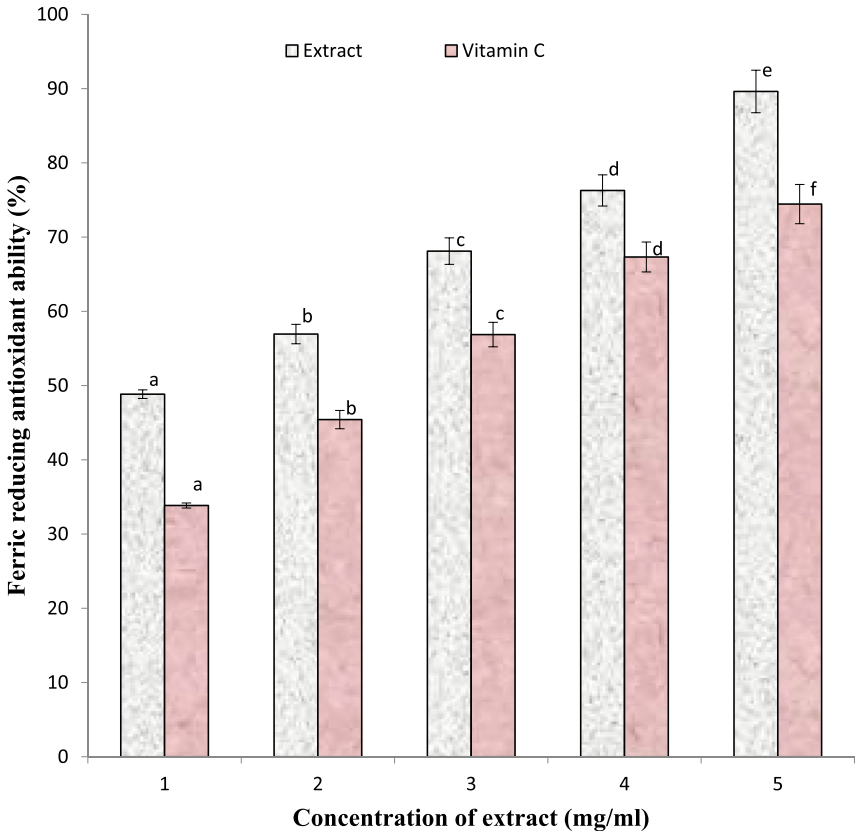

Fig. 1. Ferric reducing antioxidant properties of aqueous extract of *C. papaya* roots. Data are presented as mean ± SD of triplicate determinations (n = 3). a–f Values with different letters presented for a given concentration for each extract are significantly different from each other.

volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) are present in Table 4. Hb levels, PCV, RBCs levels, WBC, mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were markedly reduced ($p < 0.05$) in arsenic-only exposed rats comparable to control rats. Arsenic + 100 mg/kg of *C. papaya* root aqueous extract and arsenic + 150 mg/kg body weight of *C. papaya* root aqueous extract groups revealed a significant increase ($p < 0.05$) in the parameters mentioned above. Vitamin C treatment also significantly increase these parameters ($p < 0.05$).

3.8. Antioxidant status in liver

Liver antioxidant enzymes (CAT, SOD, and GPx) and liver MDA levels are revealed in Table 5. Arsenic only treated rats presented significantly lower liver CAT, SOD, GPx ($p < 0.05$) activities compared to the control rats. Administration of 150 mg/kg and 100 mg/kg body weight of *C. papaya* root aqueous extract groups significantly enhanced the activity ($p < 0.05$) of catalase, superoxide dismutase, and glutathione peroxidase. Vitamin C exposure also yielded increases of catalase, superoxide dismutase and glutathione peroxidase ($p < 0.05$), compared to the arsenic-only exposed group. The arsenic-only exposed group presented also an increase in MDA levels ($p < 0.05$), comparable to the control animals. Administration of 100 mg/kg and 150 mg/kg body weight of *C. papaya* root aqueous extract revealed significant ($p < 0.05$) decrease in the MDA levels, comparable to the arsenic-only exposed group.

3.9. Estimation of arsenic concentration in tissues

Arsenic concentrations in blood and liver tissue are presented in Table 6. Results indicated that arsenic specifically accumulated in serum and in the liver. Arsenic concentrations in the serum and tissues were considerably higher in arsenic-only exposed rats comparable to control animals ($p < 0.05$). Treatment with 100 mg/kg and 150 mg/kg body weight of *C. papaya* root aqueous extract significantly reduced its concentrations. Vitamin C treatment also produced a significant reduction in the arsenic level ($p < 0.05$).

3.10. Evaluation of chromosomal aberrations

The data for the chromosomal aberration in the bone marrow cells are displayed in Table 7. These revealed a substantial increase ($p < 0.05$) in micro nucleated polychromatic erythrocyte (MPCE) and in PCE/NCE ratios in arsenic only group comparable to control rats. However, administration with 150 and 100 mg/kg body weight of *C. papaya* root aqueous extract significantly reduced the concentrations. Moreover, vitamin C treatment also produced a significant reduction in the arsenic level ($p < 0.05$).

3.11. Evaluation of mitotic index

Results for the mitotic index in the bone marrow cells are shown in Fig. 5. It was found that the mitotic index was reduced ($p < 0.05$) in the arsenic-only exposed animals comparable to control rats. A mitotic index 60.12 ± 1.02 and 63.45 ± 1.35 was recorded in arsenic-treated rats with 100 mg/kg and 150 mg/kg body weight of *C. papaya* root aqueous extract display improved cell division in bone marrow cells of rats compared with the arsenic-only exposed

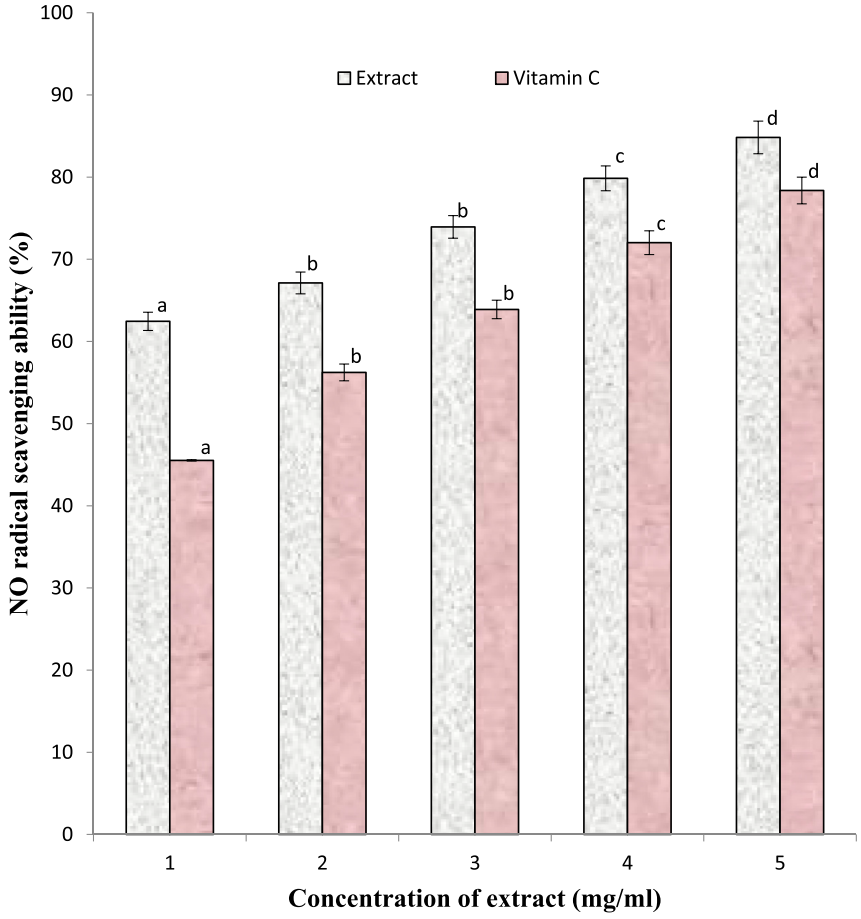

Fig. 2. NO free radical scavenging ability of aqueous extract of *C. papaya* roots. Data are presented as mean ± SD of triplicate determinations (n = 3). a–d Values with different letters presented for a given concentration for each extract are significantly different from each other.

rats (Fig. 5).

4. Discussion

In this study, we examined the anti-oxidative and geno-protective effect of *C. papaya* root aqueous extract in arsenic induced genotoxicity rat model. Sharma et al.[51] and Yadav et al.[52] stated that contact with arsenic leads to an elevation in the generation of radicals, causing biological membranes damages via high levels of malonaldehyde, protein carbonyl contents, and reduced capacity of antioxidant defense systems. According to the present data, it is evident that aqueous extracts of *C. papaya* root contain a high total phenol content as shown in (Table 1). These results are in line with previous outcomes[33]. Previous studies have strongly correlated antioxidative effect to total polyphenol contents.[33,53] Nitric oxide radical scavenging assay, ferric reducing antioxidant power and iron chelating ability are used for assessing the anti-oxidant status of compounds or plant products. Furthermore, calculated IC_{50} values were used to demonstrate the extent of the scavenging power. The lower IC_{50} values are associated with a high scavenging activity. More importantly, the aqueous extract exhibited a consistently lower IC_{50} value, for ferric reducing ability (Fig. 1), NO free radical scavenging ability (Fig. 2) and iron chelating ability (Fig. 3) suggesting that the extracts contain compounds with a high radical-quenching ability that could terminate free radical activities. This is consistent with previous studies.[54]

8-OHdG, is a novel biomarker for arsenic-induced genotoxicity and oxidative stress. It was reported that elevated levels of 8-OHdG in plasma can lead to genetic damage in arsenic exposed rats.[55] In this study, levels of 8-OHdG in plasma from arsenic only treated rats were significantly increased compared to control rats, which is similar to the reports of Nain and Smits.[55] Increased levels of 8-OHdG are due to the oxidative stress induced by the arsenic.[56] Administration of *C. papaya* root aqueous extract at 150 and 100 mg/kg body weight respectively, or vitamin C, to arsenic-exposed rats significantly reduced the 8-OHdG levels in rat plasma compared to arsenic only treated rats which might be due to the antioxidant activities of *C. papaya* root and its ability to ameliorate oxidative stress induced by arsenic.

Disturbed lipid metabolism, associated with increased low-density lipoprotein, very low density lipoprotein and total cholesterol, along with decreased high density lipoprotein -cholesterol, is usually related to arsenic toxicity.[57] Elevated levels of LDL, VLDL, atherogenic index, coronary artery index and total cholesterol are considered as foremost risk factors cardio-vascular disorders (CVD). Equally, elevated high density lipoprotein-cholesterol, that plays a crucial function in cholesterol shuttle from periphery to the liver, reduces the danger of CVD.[58] In this study, we witnessed a significantly higher concentration of serum lipids and derived AI and CRI scores with a decrease in serum high density lipoprotein-cholesterol concentration in arsenic-only exposed animals comparable to control (Table 2). Oral administration of *C. papaya* root aqueous extract at 150 and 100 mg/kg body weight along with water soluble (Vitamin C) reversed arsenic-induced dyslipidemia, reduced the degree of atherogenesis, and improved high density lipoprotein-cholesterol serum concentrations (Table 2). Results are

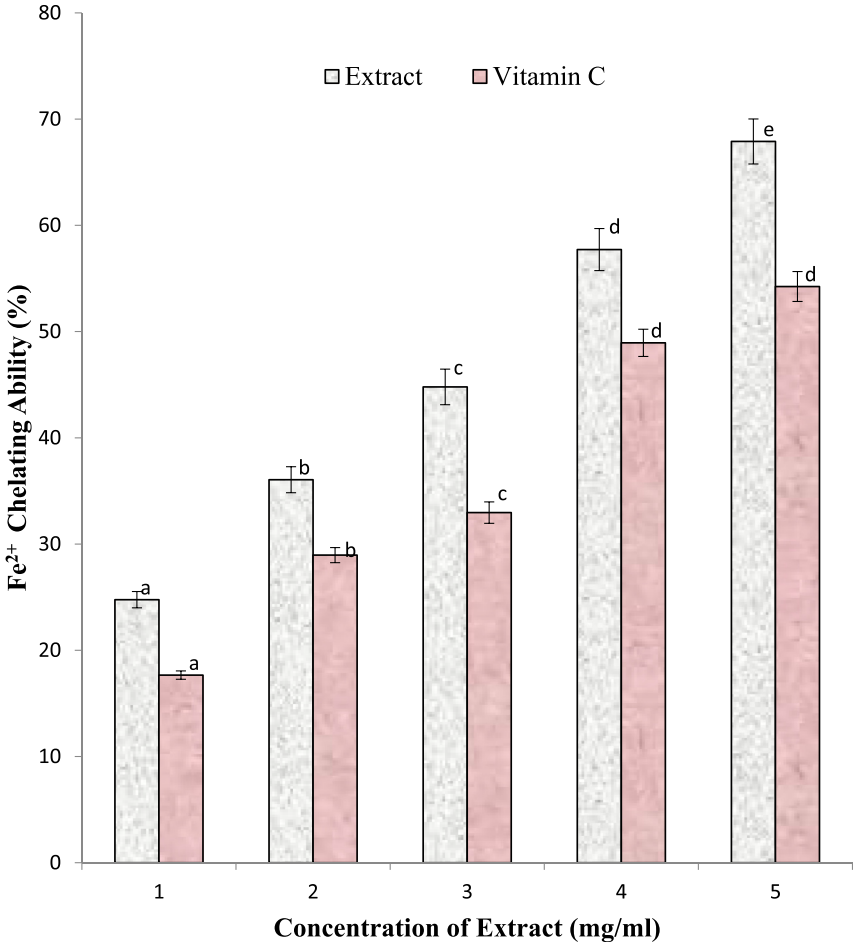

Fig. 3. Iron chelating ability of aqueous extract of *C. papaya* roots. Data are presented as mean ± SD of triplicate determinations (n = 3). a–e Values with different letters presented for a given concentration for each extract are significantly different from each other.

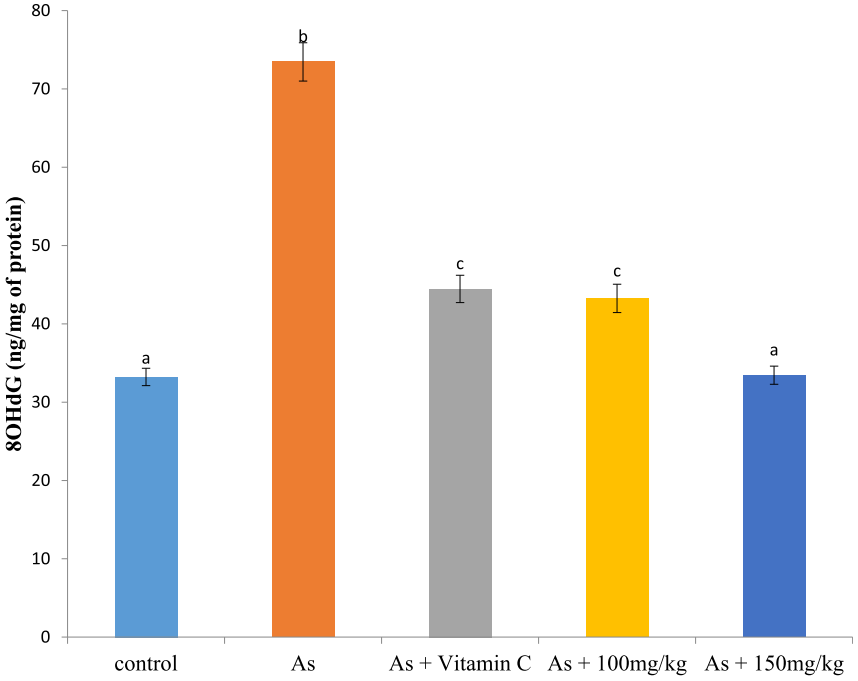

Fig. 4. Effect of aqueous extract of C. papaya roots and arsenic on the levels of plasma 8-OHdG of control and experimental rats. Values are mean ± SD for 8 rats in each group. Values not sharing a common superscript letter (a–c) differ significantly at $p < 0.05$ (Tukey's HSD multiple range post hoc test).

Table 2
Effects of administration of *Carica papaya* root aqueous extract on serum lipid profiles and atherogenic and coronary risk indices of arsenic induced genotoxicity rat serum.

Groups	TC (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	AI	CRI
Control	56.46 ± 1.14[a]	41.11 ± 1.02[a]	20.98 ± 0.11[a]	8.54 ± 1.01[a]	28.84 ± 1.01[a]	0.96 ± 0.02[a]	1.96 ± 0.05[a]
Arsenic control	98.89 ± 2.12[e]	104.26 ± 2.14[d]	74.23 ± 1.88[d]	25.85 ± 1.12[d]	9.28 ± 1.87[e]	9.66 ± 1.01[d]	10.66 ± 1.22[d]
Arsenic + vitamin C	69.98 ± 1.14[d]	73.33 ± 1.22[c]	40.39 ± 1.26[c]	12.52 ± 1.04[c]	16.29 ± 1.30[d]	3.30 ± 0.33[c]	4.30 ± 0.81[c]
Arsenic + 100 mg/kg *C. papaya* root	62.42 ± 1.12[b]	48.10 ± 1.26[b]	33.22 ± 0.04	9.82 ± 1.25[a]	26.26 ± 1.26[b]	1.38 ± 0.30[b]	2.38 ± 0.68[b]
Arsenic + 150 mg/kg *C. papaya* root	56.55 ± 1.28[a]	41.12 ± 1.16[a]	20.74 ± 0.15[a]	8.56 ± 1.01[a]	28.91 ± 1.01[a]	0.95 ± 0.01[a]	1.96 ± 0.09[a]

Data are presented as mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$). TC, Total cholesterol; TG, Triglyceride; LDL-cholesterol, Low density lipoprotein-cholesterol; HDL-cholesterol; High density lipoprotein-cholesterol, AI; atherogenic index, CRI; coronary artery index.

Table 3
Effect of administration of *Carica papaya* aqueous root extract on selected biomolecules related to of arsenic induced genotoxicity in rat serum.

Groups	AST (μ/l)	ALT (μ/l)	ALP (μ/l)	Total Bilirubin (mg/dl)
Control	98.28 ± 0.14[a]	72.20 ± 1.11[a]	188.26 ± 3.10[a]	7.41 ± 1.12[a]
Arsenic control	200.20 ± 2.22[e]	110.15 ± 1.52[e]	582.01 ± 6.17[e]	21.22 ± 1.78[d]
Arsenic + vitamin C	160.12 ± 1.43[d]	94.20 ± 1.04[d]	340.11 ± 3.54[d]	12.32 ± 1.33[c]
Arsenic + 100 mg/kg *C. papaya* root	112.14 ± 1.34[b]	82.49 ± 1.80[b]	280.13 ± 3.11[b]	9.07 ± 1.10[b]
Arsenic + 150 mg/kg *C. papaya* root	98.24 ± 1.04[a]	71.54 ± 1.02[a]	188.54 ± 3.22[a]	7.42 ± 1.54[a]

Data are presented mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$). ALT, Alanine transaminase; AST, Alanine transaminase; ALP, Alkaline phosphate.

Table 4
Effect of administration of *Carica papaya* aqueous root extract on hematological parameters related to arsenic induced genotoxicity.

Parameters	Control	Arsenic control	Arsenic + vitamin C	Arsenic + 100 mg/kg *C. papaya* root	Arsenic + 150 mg/kg *C. papaya* root
PCV (%)	67.10 ± 1.21[a]	28.10 ± 1.01[e]	52.22 ± 1.10[d]	61.25 ± 1.14[b]	67.84 ± 1.42[a]
Hb (g/dl)	15.98 ± 0.88[a]	5.14 ± 0.12[e]	10.12 ± 0.30[d]	13.72 ± 0.14[b]	15.64 ± 0.82[a]
WBC (x w^3/μl)	2.44 ± 0.12[a]	0.45 ± 0.14[e]	1.66 ± 0.18[d]	2.28 ± 0.12[b]	2.47 ± 0.24[a]
N (%)	49.12 ± 1.12	26.44 ± 1.10[e]	36.33 ± 0.11[d]	46.33 ± 0.11[b]	49.82 ± 1.45[a]
L (%)	33.22 ± 0.11[a]	19.20 ± 0.32[d]	28.23 ± 1.01[c]	30.20 ± 1.11[b]	33.48 ± 1.12[a]
M (%)	13.65 ± 0.13[a]	11.22 ± 0.04[d]	12.98 ± 0.24[c]	13.22 ± 1.22[b]	13.68 ± 1.26[a]
E (%)	4.01 ± 0.01[a]	1.92 ± 0.13[d]	2.49 ± 0.16[c]	2.98 ± 0.02[b]	3.02 ± 0.14[a]
RBC (xw^{11}/l)	3.40 ± 0.21[a]	0.50 ± 0.02[d]	1.45 ± 0.02[c]	2.84 ± 0.11[b]	3.54 ± 0.16[a]
MCHC (g/dl)	44.55 ± 1.06[a]	20.10 ± 1.04[d]	35.21 ± 1.01[c]	40.46 ± 1.02[b]	44.48 ± 1.12[a]
MCV (fl)	88.11 ± 1.10[a]	55.43 ± 1.16[e]	73.29 ± 1.10[d]	79.45 ± 1.28[b]	88.60 ± 1.09[a]
MCH (pg)	55.11 ± 1.20[a]	22.11 ± 0.16[e]	38.22 ± 0.24[d]	42.39 ± 0.56[b]	55.64 ± 1.16[a]

Data are presented mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$). Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), white blood cells (WBCs), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E).

Table 5
Effect of the administration of *Carica papaya* aqueous root extract on liver antioxidants enzymes activities and MDA levels of arsenic induced rat.

Groups	SOD (μ/mg protein)	GP_x (nm/mg/protein)	CAT (μ/mg/protein)	MDA (x10^{-8}nmd/ml)
Control	9.22 ± 1.11[a]	96.21 ± 0.15[a]	14.64 ± 1.33[a]	3.70 ± 0.02[a]
Arsenic control	2.21 ± 1.20[d]	22.28 ± 0.24[e]	3.87 ± 0.02[e]	9.87 ± 1.77[e]
Arsenic + vitamin C	6.89 ± 1.32[c]	88.35 ± 1.56[d]	9.24 ± 1.18[d]	4.34 ± 1.12[d]
Arsenic + 100 mg/kg *C. papaya* root	8.54 ± 1.09[b]	94.22 ± 0.36[b]	11.48 ± 1.45[b]	3.01 ± 0.65[b]
Arsenic + 150 mg/kg *C. papaya* root	9.28 ± 1.24[a]	96.98 ± 0.18[a]	14.98 ± 1.39[a]	3.72 ± 0.04[a]

Data are presented as mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$). SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase and MDA, malonaldehyde.

Table 6
Arsenic levels in serum and liver tissues of the studied rat.

Parameters	Control	Arsenic control	Arsenic + vitamin C	Arsenic + 100 mg/kg *C. papaya* root	Arsenic + 150 mg/kg *C. papaya* root
Liver (μg/g)	0.12 ± 0.011[a]	227.10 ± 4.41[e]	142.22 ± 5.60[d]	101.20 ± 4.04[b]	95.84 ± 3.32[a]
Serum (μg/dl)	0.065 ± 0.05[a]	156.14 ± 5.62[e]	120.12 ± 4.20[d]	100.62 ± 4.01[b]	94.64 ± 3.52[a]

Data are presented as mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$).

Table 7
Incidences of MPCE, PCE and NCE in bone marrow from the studied rat.

Group	PCE	MPCE	CPMCE	%	NCS	PCE/NCE
Control	94.32 ± 0.11[a]	65.89 ± 0.78[a]	36.56 ± 1.12[a]	5.21 ± 1.02[a]	166.27 ± 2.17[a]	6.51 ± 0.10[a]
Arsenic control	176.56 ± 3.62[e]	115.20 ± 2.62[e]	76.24 ± 2.33[e]	12.41 ± 1.98[e]	572.24 ± 4.87[e]	28.22 ± 2.52[d]
Arsenic + vitamin C	121.21 ± 2.89[d]	98.20 ± 2.14[d]	52.02 ± 2.56[d]	7.04 ± 1.47[d]	252.13 ± 3.57[d]	11.23 ± 0.59[c]
Arsenic + 100 mg/kg *C. papaya* root	101.22 ± 3.34[b]	82.45 ± 1.40[b]	40.21 ± 1.87[b]	6.12 ± 1.22[b]	192.22 ± 3.45[b]	8.11 ± 0.20[b]
Arsenic + 150 mg/kg *C. papaya* root	94.44 ± 1.10[a]	65.21 ± 1.02[a]	36.81 ± 1.11[a]	5.68 ± 1.12[a]	167.45 ± 3.01[a]	6.72 ± 1.10[a]

Data are presented as mean ± SD (n = 8). [a-e]Values with different letters along a row for a given parameter are significantly different from each other (Tukey's HSD multiple range post hoc test, $p < 0.05$). PCE; nucleated polychromatic erythrocyte, MPCE; micro nucleated polychromatic erythrocyte, NCE; normo chromatic erythrocytes.

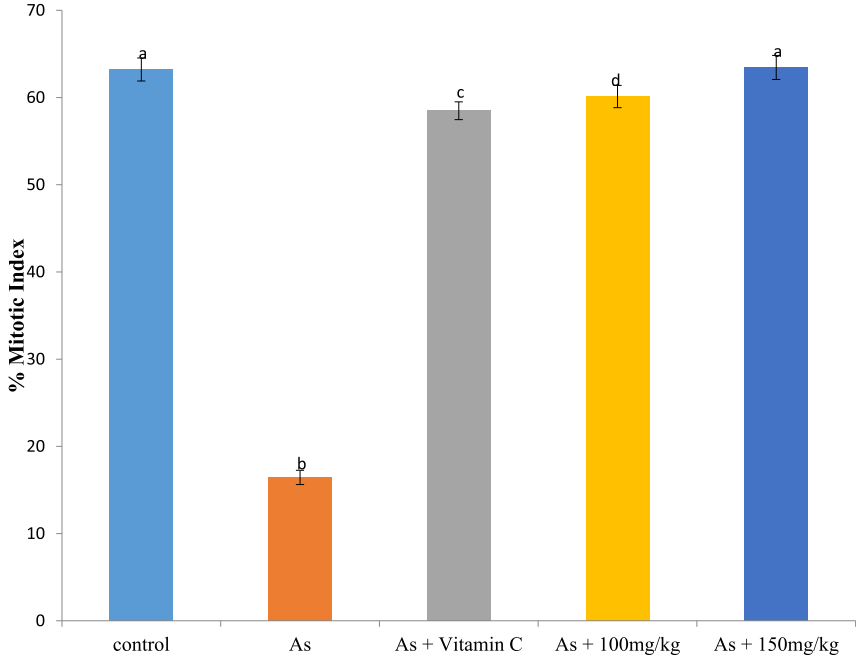

Fig. 5. Effect of aqueous extract of *C. papaya* roots and arsenic on mitotic index for bone marrow cells of control and experimental rats. Values are mean ± SD for 8 rats in each group. Values not sharing a common superscript letter (a–d) differ significantly at $p < 0.05$ (Tukey's HSD multiple range post hoc test).

in support of a variety of recent studies, which yielded similar outcomes.[59]

High levels of serum transaminase, alanine aminotransferase (ALT), aspartate aminotransaminse (AST), and alkaline phosphatase (ALP) are well established as markers of hepatic impairment, related for example to fatty liver disease.[60] Treatment with *C. papaya* root aqueous extract at 150 and 100 mg/kg body weight (Table 3) significantly reduced ($p < 0.05$) ALT, AST, ALP and bilirubin levels, suggesting that *C. papaya* root aqueous extract ameliorates arsenic-induced hepatocyte injury in rats. These results are also in support of earlier research outcomes.[61–63] It was suggested that hepatocyte injury following metal exposure as a result of binding to the biomembranes and buildup within the mitochondria, resulting in the breakdown of the mitochondrial membrane, followed by apoptosis.[64,65]

Blood assessment could be a reliable approach of evaluating the health condition of animals because it plays a significant role in the maintaining of the physiological, pathological, and nutritive condition of organisms.[66,67] Evaluation of haematological variables may be used to verify the extent of harmful assaults on blood components.[68–70] It can even be used to rationalize blood-related functions of biochemical combinations or extracts.[71,72] In arsenic only treated rats, significant ($p < 0.05$) reductions in haemoglobin (Hb) levels, white blood cells (WBCs), red blood cells (RBCs), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), neutrophils (N), lymphocyte (L), monocytes (M) and eosinophils (E) were witnessed throughout the analysis of the haematological parameters (Table 4). Considerable elevation of Hb levels, RBCs, WBCs, PCV, MCHC, MCV, MCH, N counts, L, M, and E counts occurred upon administration with *C. papaya* root aqueous extract at 100 mg/kg and 150 mg/kg doses and values attained were significantly different ($p < 0.05$) compared to that obtained of the arsenic only treated group. The same trend was seen in the levels of parameters mentioned above for arsenic + vitamin C (200 mg/kg body weight) treated rats compared to arsenic only treated rats. This might be due to the antioxidant activity of *C. papaya* root. Thus, it was established that arsenic-induced animals revealed anomalies in the studied hematological parameters. A number of these anomalies could be due to damage to the red blood cells resulting in the low haemoglobin levels observed with simultaneous drop in the red blood cell and packed cell volume[73–75]; Low levels of red blood cells, haemoglobin and hematocrit might suggest anemia.[73–75] However, immunomodulatory effects and confined toxicity might occur in the neutrophils and lymphocytes of the arsenic-alone exposed animals. Treatment with *C. papaya* root aqueous extract has beneficial effects by causing modifications in the haematological variables of the arsenic-induced rats. Hence, elevation in red blood cells by the extract is a sign of its beneficial effect on arsenic-induced anemia.

Elevated oxidative stress was found to play an important effect within the induction of programmed cell death. Amplified production of radicals, related to elevated oxidative stress, was found to be involved in arsenic toxicity.[51] Enzymatic antioxidants such as catalase and superoxide dismutase are the primary line of defense towards oxidative stress upon arsenic exposure. These two enzymes also are basic defense markers of the antioxidant system that combats radicals created following arsenic treatment. Suppressed activity of catalase and superoxide dismutase in arsenic-treated rat's reveals correlation to a higher generation of superoxide radicals[76] and inadequate demand of NADPH, with the latter being needed for the stimulation of catalase from its inactivated form.[77] Consistent with this, decreased levels of main marker enzymes, such as catalase, superoxide dismutase and glutathione peroxidase along with increased malonaldehyde evident by increased MDA levels within the arsenic only treated rats (Table 5) was observed, a sign of arsenic-induced oxidative stress. Administration of *C. papaya* root aqueous extract at 100 mg/kg and 150 mg/kg body weight, correlated with considerably increased antioxidant enzymes, as well as with reduced MDA levels, signifying the capability of *C. papaya* root aqueous extract to reduce oxidative stress in arsenic-exposed animals.

Statistics generated revealed that arsenic accumulated preferentially in the liver and serum. Arsenic concentrations in the serum and tissues (Table 6) were considerably higher ($p < 0.05$) in arsenic-only exposed rats. However, treatment with *C. papaya* root aqueous extract at 150 and 100 mg/kg body weight considerably decrease the levels of arsenic in the tissues. Vitamin C administration also reduced the levels of arsenic in the tissues compared to the arsenic-only exposed rats. These results are in accordance with results reported by Miltonprabu and Sumedha,[78]; which showed that arsenic dispersal in tissues depends on the route of administration and its form.

Data obtained in this work show a significantly increased genetic damage within the bone marrow cells, following arsenic exposure. Fractions of anomalous cells within bone marrow from exposed rats revealed a considerable increase ($p < 0.05$) comparable to normal rats. In the bone marrow cells, there was a progressive increase in the proportion of MPCE and PCE/NCE ratios in arsenic only treated rats comparable to cells from the control rats. However, treatment with *C. papaya* root aqueous extract at 100 and 150 mg/kg body weight significantly decreased percentages of MPCE and PCE/NCE ratios. Also, vitamin C treatment group significantly reduced the percentage of MPCE and PCE/NCE ratios as well. Also, a significant elevation ($p < 0.05$) in micronuclei was witnessed in cells derived from arsenic-only exposed rats comparable to cells from control rats. Treatment with *C. papaya* root aqueous extract at 100 mg/kg and 150 mg/kg body weight and vitamin C (200 mg/kg) significantly decreased chromosomal aberrations and, micro nuclei induction with an increase in the mitotic index. The types of cytogenetic damage detected in this work may be ascribed to many modes of arsenic toxicity such as inhibition of enzymes concerned in the repair of DNA and gene expression,[79] the increase of ROS levels capable of inflicting damage on biopolymers[80–82] or the induction of gene expression resulting in modification in repair mechanisms maintaining DNA integrity.[56]

5. Conclusion

These results show that arsenic includes pro-oxidative and genotoxic effects, as estimated by the bone marrow chromosomal aberrations and micronuclei tests in rats, and that *C. papaya* root aqueous extract at the tested doses reverses the pro-oxidative and genotoxic effects inflicted by arsenic.

Conflict of interest

The authors declare that there are no competing interests.

Authors' contributions

OAO: designed the experiment; ABO, OA and BEO: conducted analysis and drafting of the manuscript; IO, AI performed the experiments; BOA and OAO: collection of data; analysis and evaluation of data; statistical analysis. All authors read and approved the final version of the manuscript.

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