Journal of Applied Sciences

ISSN 1812-5654

science alert

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OPEN ACCESS Journal of Applied Sciences

ISSN 1812-5654
DOI: 10.3923/jas.2023.143.153

Research Article
Male Reproductive Toxicity and Modulation of Enzyme Activities by the Extracts of *Carica papaya* and *Bridelia ferruginea*

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Abstract

Background and Objective: *Carica papaya* and *Bridelia ferruginea* are traditionally used in the prevention and management of several diseases. However, limited information exists on the potential adverse effects of these plant extracts on germ cells. This study aimed at investigating the reproductive toxicity of the aqueous extracts of *C. papaya* (leaves and unripe pulp) and *B. ferruginea* (stem bark) using a murine sperm morphology assay. Also, the serum biochemical assay of the experimental mice was carried out using selected serum enzymes as biomarkers to evaluate the safety or toxicological potentials of these extracts. **Materials and Methods:** The reproductive toxicity of the aqueous extracts of *C. papaya* (unripe pulp and leaves) and *B. ferruginea* (stem bark) in male mice was evaluated using sperm morphology assay and testes histopathology at 200, 100 and 50 mg kg^{-1} body weight of each extract. Assessment of selected serum enzymes and oxidative stress biomarkers was also carried out with the analysis of selected sex hormones. Doses were orally administered to the mice daily for seven days. **Results:** Sperm morphology revealed abnormal sperm cells in the exposed mice in all the doses of the extracts. However, the extract of *B. ferruginea* significantly ($p<0.05$) induced sperm abnormality in a dose-dependent manner. Furthermore, all the doses of *C. papaya* leaf extract also induced significant abnormal sperm cells. Testes histopathology showed alterations of the seminiferous tubule of the exposed mice with varying degrees and types of lesions. The hepatic damage markers (aminotransferases) and renal damage (alkaline phosphatase) significantly increased in mice exposed to the extract of *B. ferruginea* stem bark. Serum alkaline phosphatase activity was increased in the mice exposed to 100 mg kg^{-1} body weight *C. papaya* leaf extract. However, a significant decrease was observed in the catalase and superoxide dismutase activities in the treated mice. A significant increase in follicle stimulating hormone and total testosterone and a decrease in luteinizing hormone was also observed in the exposed mice. **Conclusion:** Therefore, the findings of the present study indicated that the aqueous extracts of *C. papaya* leaf and *B. ferruginea* stem bark could induce toxicity in the germ cells of mice.

Key words: Sperm morphology, *Carica papaya*, *Bridelia ferruginea*, reproductive toxicity, sex hormones

Citation: Omotade, O., A. Okunola, A. Mary, O. Samuel and O. Tobi *et al*., 2023. Male reproductive toxicity and modulation of enzyme activities by the extracts of *Carica papaya* and *Bridelia ferruginea*. J. Appl. Sci., 23: 143-153.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Plants play vital roles in the advancement of human culture and traditional medicine. They are believed to be rich sources of folk medicines, hence, plants are major raw materials for the production of most contemporary medicines[1]. Indeed, the majority of the world's population depends on plants for their primary health care[2]. In Nigeria, ethnopharmacological uses of plants are very common. Various plant parts such as the leaves, whole fruits, pulp, stem, stem bark and roots are extensively used. Aside from the therapeutic properties exhibited by these plants, they are easily accessible and are believed to be much safer than modern synthetic drugs. This has led to increased use of phytochemicals from plants as a potent source of therapeutic ingredients.

Carica papaya Linn. (Family: Caricaceae) is a delicious fruit with fragrant undertones and a characteristic pleasant aroma. It has a soft texture with butter-like consistency when ripe, with its taste and sweetness increasing with the ripening process of the fruit[3]. *Carica papaya* is used as medicine, cooking aid and food[4]. It has been used extensively in traditional medicine for many ailments. The fruit, seeds and leaf contain many biologically active compounds and antioxidants with broad-spectrum antimicrobial activity[5]. Its aqueous leaf extract can significantly decrease blood glucose levels, promote wound healing in diabetic rats and lower blood pressure[6]. This aqueous extract of *C. papaya* leaf is also commonly consumed daily in Nigeria as an anti-malarial decoction. *Carica papaya* fruit is often eaten raw when ripe without seeds or skin, while the unripe fruit is eaten raw and cooked and can also be soaked in water to extract the bioactive contents for medicinal purposes[7]. Unripe *C. papaya* fruit is rich in pectin, an ingredient for making jellies[8]. It contains a milky juice that has a protein-digesting enzyme known as papain. Papain is a proteolytic enzyme with great resemblance as a digestive enzyme to pepsin, an animal enzyme. The juice has been used to manufacture meat tenderizers and prepare different remedies for ingestion. Previous phytochemical screening and chemical analysis of the unripe pulp of *C. papaya* revealed the presence of cardenolides, saponins, manganese, calcium, sodium, phosphorus, iron, magnesium, copper, zinc and potassium in substantial amounts. Cardenolides and saponins present in this plant are most likely responsible for their astringent action in several therapeutic uses[9].

Bridelia ferruginea (Family: Phyllanthaceae) is a shrub, sometimes with spiny branches growing up to 8-15 m in height. Although grown mostly in the wild, it is a very common medicinal plant in Nigeria, especially among the people of the Southwestern part of the country. *Bridelia ferruginea* parts such as the leaves, leafy twigs and bark are all used for ethnopharmacological purposes. The plant has hydrolipidemic and hypolipidemic properties and as such can be used in the treatment of *Diabetes mellitus*. Extract from *B. ferruginea* also has anti-inflammatory, anti-malaria and antibacterial properties[10]. The medicinal activity of *B. ferruginea* might be a result of the presence of saponosides and tannins in it. Aqueous extracts and infusions from the leaves, leafy twigs and bark are commonly used in the treatment of arthritis and rheumatic pains, urethral discharges and boils, dysentery and diarrhea, toothache and fever. The root decoction also has similar use while a decoction of the bark is used for treating toothache[11].

Despite the many beneficial effects of using medicinal plants, yet, many detrimental health effects have been documented. Cytotoxic safety of plant materials, especially medicinal plants, is a significant public health issue, in other to ensure human safety. Reproductive toxicity of plant extracts is an important aspect of toxicity that should be carefully and appropriately investigated to validate or invalidate existing literature on medicinal plants. Plant extracts are assumed to be safe for use, but this is not always the case. Usually, toxicological studies on plants are mostly based on acute and sub-chronic tests or observation of general behavior and clinical signs, however, this might not be sufficient to ascertain the reproductive health risks associated with the use of these plants.

A sperm morphology test is a test that easily quantitates and gives a direct measurement of the exposed animal's sperm quality. It is technically simple, cheap and relatively fast in comparison with the majority of other short-term *in vivo* tests[12]. In sperm morphology and structure assessment, the head shape and size, tail and mid-piece are observed. Also, information about the sperm acrosomes and cell membranes can be added. The final result is calculated as a percentage of morphologically normal sperm cells obtained from the control group. Normally, some amount of sperm cells with morphological abnormalities are always present in an ejaculate, however, when there is a large percentage of abnormally shaped sperm cells, it may affect fertility[13,14].

In spite of the wide usage for medicinal benefits of these two plants (*C. papaya* and *B. ferruginea*), very few studies on their potential toxicity especially about male reproductive toxicity have been reported. There is a need for more data using different animal models to elucidate the potential reproductive toxicity of these medicinal plants and to make scientifically informed decisions about their regular use.

Hence, this study aimed at investigating the reproductive toxicity of the aqueous extracts of *C. papaya* (leaves and unripe pulp) and *B. ferruginea* (stem bark) using a murine sperm morphology assay. Also, the serum biochemical assay of the experimental mice was carried out using selected serum enzymes as biomarkers to evaluate the safety or toxicological potentials of these extracts.

MATERIALS AND METHODS

The study was carried out from November, 2021 to October, 2022.

Experimental plant: The fresh stem of *B. ferruginea* and leaves and unripe pulp of *C. papaya* were obtained from a farm in Ado-Ekiti, Ekiti State, Nigeria and were subsequently sent for identification and authentication at Ekiti State University Herbarium.

Sample preparation: The fresh leaves and unripe pulp of *C. papaya* and fresh stem of *B. ferruginea* were separately washed with distilled water before they were air-dried and pulverized. Five grams of each of the samples were weighed, mixed and soaked overnight in 100 mL of distilled water. The extracts were filtered (Whatman® No. 1) and the resulting filtrates were separated for use, while the residue was discarded. The method of preparation of the aqueous extracts of these two plants was chosen to simulate the method used by the indigenes who consumed these plants regularly.

Phytochemical screening: The ground plant material (400 g) of each plant was boiled for 10 min in 4 L of distilled water and then filtered (Whatman® No. 1). A rotary evaporator (Model RE52A, China) was then used to concentrate the filtrate into a semi-solid brown gelatinous extract at 40°C under reduced pressure and then refrigerated at 4°C throughout the period of the study. The extract was screened for the presence of cardiac glycosides, resin, alkaloids, flavonoids, saponins, anthraquinones, phenols, steroids and tannins according to standard procedures[15-17].

Animal treatment: Seventy-seven male Swiss albino mice (*Mus musculus*) 9-10 weeks old weighing 35-45 g, were purchased from the Department of Physiology's Animal Breeding Unit of the University of Ibadan, Nigeria. They were kept for 2 weeks to acclimatize them in the Department of Biochemistry's animal house, at Ekiti State University, Nigeria. The mice were fed with standard pelletized feed (Top Feed pelleted feed®) and clean drinking water *ad libitum* till the termination of the experiment. The mice were carefully handled as stipulated in standard guidelines[18].

Sperm morphology abnormalities assay: Seven mice each were randomly grouped into eleven groups as follows. The doses were chosen based on our preliminary acute toxicity study (data not shown).

- Group 1 (Negative control) was orally exposed to distilled water
- Group 2 (Positive control) was exposed to cyclophosphamide (20 mg kg^{-1} b.wt.)
- Group 3 was orally exposed to 50 mg kg^{-1} b.wt., aqueous extracts of *C. papaya* leaf
- Group 4 was orally exposed to 100 mg kg^{-1} b.wt., aqueous extracts of *C. papaya* leaf
- Group 5 was orally exposed to 200 mg kg^{-1} b.wt., aqueous extracts of *C. papaya* leaf
- Group 6 was orally exposed to 50 mg kg^{-1} b.wt., aqueous extracts of *C. papaya unripe* pulp
- Group 7 was orally exposed to 100 mg kg^{-1} b.wt., aqueous extracts of *C. papaya* unripe pulp
- Group 8 was orally exposed to 200 mg kg^{-1} b.wt., aqueous extracts of *C. papaya* unripe pulp
- Group 9 was orally exposed to 50 mg kg^{-1} b.wt., aqueous extracts of *B. ferruginea* stem bark
- Group 10 was orally exposed to 100 mg kg^{-1} b.wt., aqueous extracts of *B. ferruginea* stem bark
- Group 11 was orally exposed to 200 mg kg^{-1} b.wt., aqueous extracts of *B. ferruginea* stem bark

The mice were orally exposed to 0.4 mL of each of the concentrations per group for seven consecutive days. Five of the seven mice per group were sacrificed on the 35th day since about 34.5 days were required for spermatogenesis to be complete in mice[19]. The procedures of Wyrobek *et al.*[20] and Alabi *et al.*[14] were followed to study the induction of morphologically abnormal sperm morphology. After sacrificing the mice through cervical dislocation, their epididymides were removed surgically. The sperm cells obtained from the caudal epididymis of each testis were made into separate suspensions after mincing the caudal epididymis in normal saline. The sperm cells were stained for 45 min in 1% eosin Y, smeared on grease-free slides and then air-dried before the microscopic examination at 1000x under oil immersion. A total of 2000 sperm cells per mouse were analyzed for abnormal morphology using the criteria of Wyrobek and Bruce[12].

Biochemical analysis: The rest of the mice in each group in the sperm morphology assay were used for this study. The animals were sacrificed and their testes were excised, weighed and rinsed in 1.15% Potassium Chloride (KCl). Part of the testes was then homogenized in 0.1 M ice-cold phosphate buffer (pH 7.4) and centrifuged at 10000 rpm for 15 min at 4°C to obtain a clear supernatant. The method of Gornall *et al.*[21] for the quantification of the total protein in the homogenates using the Biuret reagent was used. Total protein was spectrophotometrically measured at 540 nm using Bovine Serum Albumin as standard. Activities of superoxide dismutase (SOD), Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), catalase (CAT) and Alanine aminotransferase (ALT) were determined using commercial kits (Fortress Kit, England).

Histopathology of testes: The best part of the testes used for the biochemical assay was used for this study. Fixation of the testes in bouin's fluid was carried out for 48 hrs before dehydration in ascending ethanol concentrations. The testes were cleared in xylene and paraffin wax was used for embedding for 6 hrs. The embedded testes were serially sectioned using a rotary microtome (Microm, Walldorf, Germany) to about 4 µm thickness and placed on clean microscope slides, stained with hematoxylin-eosin and observed using a light microscope (Microscope Leica DM6000 B, Germany) at 400x.

Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH) and total testosterone (TT) analyses: The blood of the same mice used for sperm morphology assay was 21 collected and the serum was isolated after centrifugation at 3000 g for 5 min. Commercially available kits (Beckman Coulter, Inc., USA) were used for the quantification of the concentrations of LH, FSH and TT using an automated Unicel Dxl 800 Access Immunoassay System and Chemiluminescent Immunoassay.

Ethical consideration: Animals were cared for in accordance by the ethical standards of the Ethics Committee for Animal Use of the Federal University of Technology, Akure, Nigeria (TNC 121) and the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2021).

Statistical analyses: The data obtained were analyzed using SPSS® 22.0 and represented as Mean±Standard error of the mean. Significance among the groups was determined using ANOVA and Dunnett t-test with a 0.05 probability level recorded as significant.

RESULTS

Phytochemical screening: The result of the phytochemical screening showed that the extract contained secondary metabolites such as saponins, cardiac glycosides, anthraquinone, alkaloids, glycosides and flavonoids. The phytochemical analysis of the *C. papaya* leaves and pulp showed the presence of flavonoids, tannins, saponins and alkaloids such as dehydrocarpaines I and II, pseudocarpaine and carpaine. Similarly, the extract of the stem bark of *B. ferruginea* contained steroids, alkaloids, cardiac glycosides, tannins and saponins.

Sperm morphology assay: The summary of the sperm cell abnormalities in mice treated with aqueous extracts of fresh leaves, the unripe pulp of *C. papaya* and the fresh stem of *B. ferruginea*. The three aqueous extracts showed three different results in the induction of abnormal sperm cells as shown in Table 1.

Table 1: Summary of the morphologically abnormal sperm cells induced in mice treated with aqueous extracts of fresh leaves and unripe pulp of *Carica papaya* and fresh stem of *Bridelia ferruginea*

Treatment	Mean±SEM	Frequency of abnormality (%)
Negative control	105.67±2.73	5.28
Positive control	937.33±4.81*	46.87
***Carica papaya* (leaf)**		
50 mg kg^{-1}	351.66±12.25*	17.58
100 mg kg^{-1}	176.00±15.56*	8.80
200 mg kg^{-1}	236.00±1.73*	11.80
***Carica papaya* (pulp)**		
50 mg kg^{-1}	104.67±4.84	5.23
100 mg kg^{-1}	111.33±4.10	5.57
200 mg kg^{-1}	227.33±7.51*	11.37
***Bridelia ferruginea* (stem bark)**		
50 mg kg^{-1}	653.00±19.66*	32.65
100 mg kg^{-1}	359.00±2.52*	17.95
200 mg kg^{-1}	182.67±7.22*	9.13

Negative control: Distilled water, Positive control: Cyclophosphamide, SEM: Standard error of mean, *Significant at $p<0.05$ compared to the negative control and Data are expressed as Mean±SEM (n = 5)

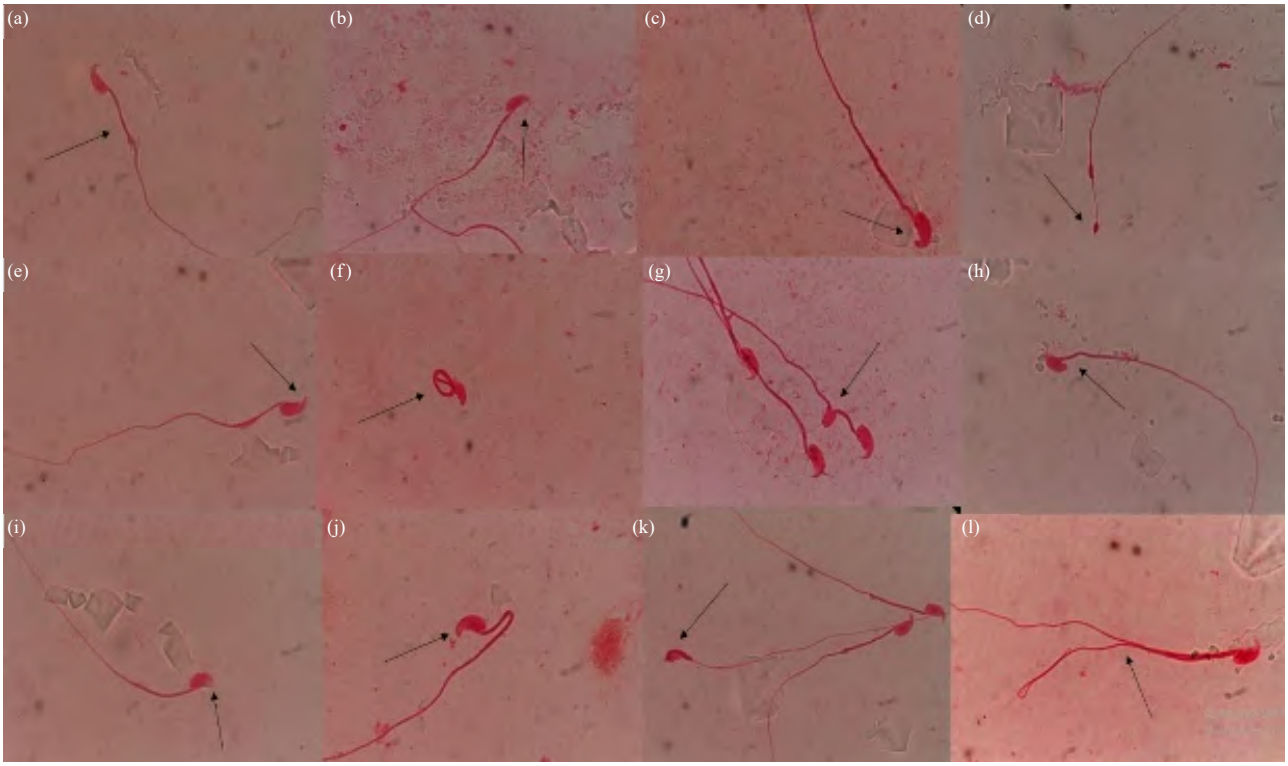

Fig. 1(a-l): Representative abnormal sperm morphologies induced in mice exposed to aqueous extracts of *Carica papaya* and *Bridelia ferruginea*, (a) Normal sperm cells, (b) Knobbed hook, (c) Swollen hook, (d) Pinhead, (e) Short hook, (f) Folded sperm, (g) Fused neck sperm, (h) Tail at the wrong angle, (i) No hook, (j) Banana head, (k) Amorphous head and (l) Double tail sperm
1% Eosin Y stain and ×100

However, the aqueous extract of *C. papaya* leaf showed significant induction of abnormal sperm cells at the three doses tested with the 50 mg kg^{-1} b.wt., inducing the highest abnormalities (351.66±12.25), followed by the 200 mg kg^{-1} b.wt. (236.00±1.73). Also, the extract of *C. papaya* unripe pulp caused a dose-dependent increase in sperm cells with morphological abnormalities, which was only significant ($p<0.05$) at 200 mg kg^{-1} b.wt. Similarly, the extract of *B. ferruginea* caused a significant ($p<0.05$) dose-dependent increase in sperm cells with morphological abnormalities in comparison with the negative control, with the lowest dose (50 mg kg^{-1} b.wt.) causing the highest abnormality, followed by the 100 mg kg^{-1} b.wt.

The positive and negative controls had 46.87 and 5.28% of sperm cells with morphological abnormalities, respectively. The highest percentage of sperm abnormality was induced by 50 mg kg^{-1} b.wt., of *B. ferruginea* stem bark (32.65%), while the lowest sperm abnormality was induced by 50 mg kg^{-1} b.wt., of *C. papaya* unripe pulp. Different types of abnormal sperm cells such as sperm cells with amorphous head, knobbed hook, short hook, tail attached wrongly, long hook, banana head, folded sperm cells and fused sperm cells were observed in the exposed mice (Fig. 1). Fused sperm cells have the least occurrence while sperm cells with amorphous heads had the highest frequency of occurrence (Fig. 2).

Biochemical analysis: Alteration in the activities of SOD, ALP, ALT, CAT and AST was observed in mice exposed to the aqueous extracts of *C. papaya* leaf and unripe pulp and *B. ferruginea* stem bark in comparison with the negative control (Table 2). Only the aqueous extract of *B. ferruginea* induced a significantly ($p<0.05$) increased ALT and AST activities (200 mg kg^{-1} b.wt., for AST and 100 and 200 mg kg^{-1} b.wt., for ALT), however, all three extracts induced a significant decrease in CAT activity. Similarly, a significant ($p<0.05$) decrease in the activity of ALP was observed at the 200 mg kg^{-1} b.wt., of *C. papaya* leaf and *B. ferruginea* stem bark, while SOD activity significantly ($p<0.05$) decreased at 200 mg kg^{-1} b.wt., of *B. ferruginea* and 100 mg kg^{-1} b.wt., of *C. papaya* leaf and unripe pulp.

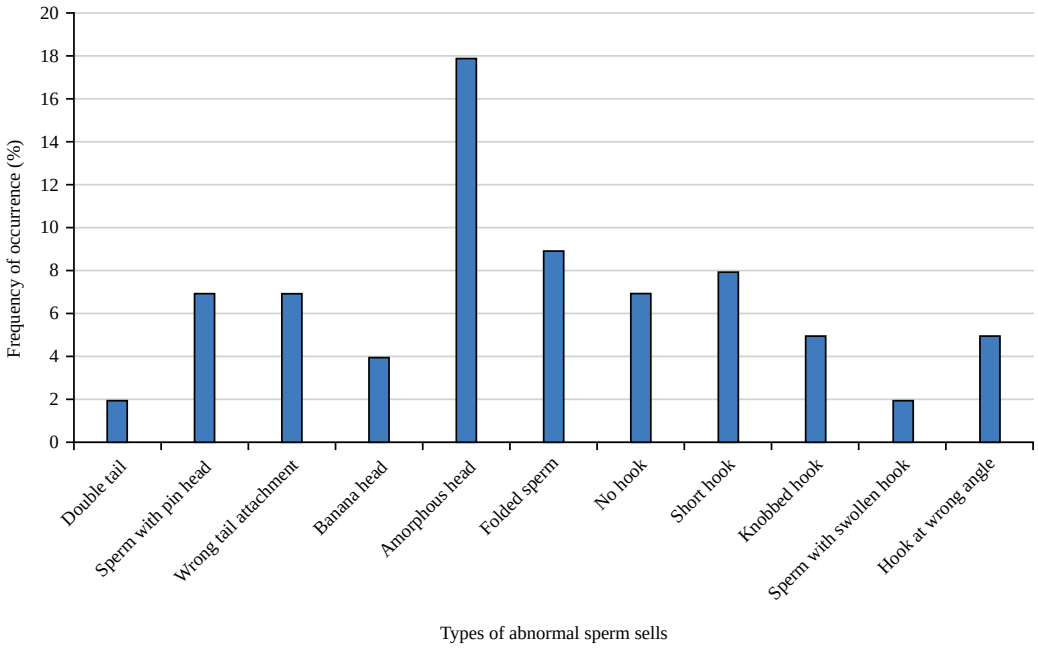

Fig. 2: Percentage frequencies of each type of sperm aberration induced in mice exposed to aqueous extracts of *Carica papaya* and *Bridelia ferruginea*

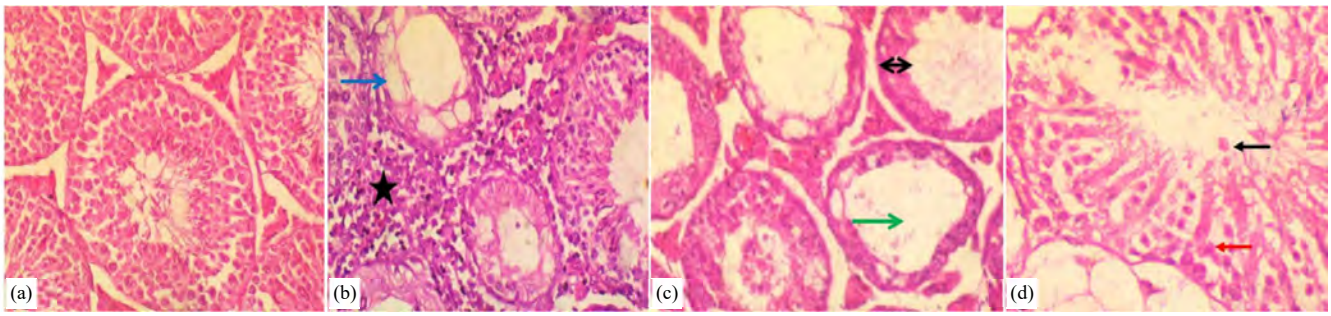

Fig. 3(a-d): Testes histopathological assessment in mice treated with the aqueous extracts of *Carica papaya* and *Bridelia ferruginea* (a) Negative control: Seminiferous tubule with no visible lesions, (b) *Carica papaya* pulp: Seminiferous epithelium vacuolation (blue arrow) and interstitial cell proliferation resulting in congestion of the interstitial space (black star), (c) *Carica papaya* leaf: Decrease in the diameter of the seminiferous epithelium (double-ended arrow) and increase in luminal width (green arrow) and (d) *Bridelia ferruginea* stem: Necrosis of spermatogonia (black arrow), apical sloughing of germinal epithelium (red arrow)
Magnification: x400, Eosin and hematoxylin stains

Histopathology of the testes of the exposed mice: The histopathological observations of the testes of mice exposed to the aqueous extracts of *C. papaya* leaf and unripe pulp and *B. ferruginea* stem bark as shown in Fig. 3. Examination of the negative control mice testes showed no visible lesions of the cellular architecture of the testicular tissues. However, the mice treated with *C. papaya* unripe pulp showed the presence of interstitial cell proliferation causing congestion of the interstitial space and seminiferous epithelium vacuolation. The testes of mice treated with *C. papaya* leaf on the other hand showed an increased luminal width and a decreased diameter of the seminiferous epithelium while the group treated with *B. ferruginea* stem showed germinal epithelium apical sloughing and spermatogonia necrosis.

Table 2: Effect of aqueous extracts of fresh leaves and unripe pulp of *Carica papaya* and fresh stem of *Bridelia ferruginea* on some biochemical parameters in mice

Treatment	AST (UI)	ALT (UI)	ALP (UI)	CAT (U mg^{-1} protein)	SOD (U mg^{-1} protein)
Negative control	11.06±0.39	9.19±0.73	48.41±0.86	0.08±0.04	0.003±0.001
Positive control	17.25±0.32*	15.40±0.82*	63.89±3.12*	0.05±0.01*	0.001±0.000*
***Carica papaya* (leaf)**					
50 mg kg^{-1}	10.62±0.61	8.77±0.88	45.83±4.62	0.02±0.10*	0.002±0.000
100 mg kg^{-1}	11.75±0.54	9.07±0.86	47.61±1.71	0.02±0.00*	0.001±0.006*
200 mg kg^{-1}	11.43±0.54	10.29±0.51	53.86±0.51*	0.01±0.01*	0.002±0.000
***Carica papaya* (pulp)**					
50 mg kg^{-1}	10.38±0.56	9.13±0.37	47.02±1.24	0.02±0.01*	0.002±0.005
100 mg kg^{-1}	11.00±0.53	8.18±0.49	47.42±1.98	0.02±0.01*	0.001±0.028*
200 mg kg^{-1}	11.00±0.84	9.19±0.43	47.02±1.03	0.02±0.27*	0.002±0.001
***Bridelia ferruginea* (stem bark)**					
50 mg kg^{-1}	10.81±0.38	10.81±0.47	45.44±1.80	0.04±0.01*	0.005±0.000
100 mg kg^{-1}	12.63±0.23	12.60±0.26*	48.61±0.79	0.03±0.01*	0.004±0.001
200 mg kg^{-1}	14.13±0.30*	14.87±0.21*	58.13±0.79*	0.03±0.00*	0.001±0.000*

Negative control: Distilled water, Positive control: Cyclophosphamide, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, CAT: Catalase, SOD: Superoxide dismutase, *Significant at p<0.05 compared to the negative control and Data are expressed as Mean±SEM (n = 5)

Table 3: Serum concentration of total testosterone (TT), Follicle Stimulating Hormone (FSH) and luteinizing hormone (LH) in mice exposed to different concentrations of *Carica papaya* and *Bridelia ferruginea*

Concentration (mg kg^{-1})	TT (ng mL^{-1})	FSH (mIU mL^{-1})	LH (mIU mL^{-1})
***Carica papaya* (leaf)**			
NC	5.63±0.02	0.11±0.20	1.98±0.04
50	9.83±0.43*	0.19±0.10*	1.15±0.55
100	7.64±0.33	0.15±0.81	1.07±0.20*
200	8.73±0.10*	0.18±0.09*	0.79±0.01*
CYP	10.28±0.51*	0.21±0.05*	0.29±0.11*
***Carica papaya* (pulp)**			
50	5.98±0.22	0.12±1.01	1.86±0.05
100	5.96±0.31	0.12±0.92	1.89±0.02
200	6.38±0.51*	0.16±0.21*	1.28±0.47*
***Bridelia ferruginea* (stem bark)**			
50	11.05±0.01*	0.19±0.20*	0.38±0.31*
100	12.18±0.28*	0.21±0.07*	0.31±0.05*
200	13.42±0.81*	0.24±0.17*	0.27±0.01*

NC: Distilled water, CYP: Cyclophosphamide (positive control), *Significant at p<0.05 compared to the negative control, Data are expressed as Mean±SEM (n = 5)

Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH) and total testosterone (TT) analyses: The level of LH, FSH and TT recorded in the serum of exposed mice was shown in Table 3. A significantly (p<0.05) increased TT and FSH were recorded in exposed mice at all the concentrations of *C. papaya* leaf and *B. ferruginea* stem tested and at the highest concentration of the *C. papaya* pulp in comparison to the negative control group.

However, mice treated with *C. papaya* leaf and *B. ferruginea* stem showed a significantly (p<0.05) reduced LH at all concentrations except for the *C. papaya* pulp treated mice which showed significant reduction only at the highest concentration of 200 mg kg^{-1} when compared with the negative control group as shown in Table 3.

DISCUSSION

The data of the present study showed that *C. papaya* leaf and *B. ferruginea* extracts are capable of inducing reproductive toxicity and also modulate enzyme activities in exposed mice. From the data recorded in the sperm morphological abnormality test, sperm abnormalities were induced in mice treated with the three plant extracts. This result indicated that the extracts are capable of inducing reproductive toxicity in mice. The potential of the extracts to cause alteration to the process of spermatogenesis is indicated by the different types of abnormal sperm cells including sperm cells with amorphous heads, knobbed hooks, short hooks, tails attached wrongly, long hooks, banana heads, folded sperm cells and fused sperm cells that were observed in the exposed mice. The epithelial cells involved in spermatogenesis are most times the target cells of the different phytochemicals contained in plant extracts[22], as with other xenobiotics[23]. Alteration of the seminiferous epithelium and the process of gametogenesis by genotoxic substances is reflected in the number of genetically defective abnormal sperm cells recorded[24]. While there are debates around the effect of defects of sperm cell morphology on fertilization or development of the embryo, evidence of the development of abnormal karyotypes with structural aberrations that are

believed to have developed from abnormal spermatozoa exists[25,26]. Therefore, the ability of these plant extracts to induce sperm cell abnormality is of public health concern, especially among the populace who depend on these plants for medicine.

Furthermore, all the doses of the stem bark extract of *B. ferruginea* used were toxic to mice germ cells. A lower dose of the extract induced the highest abnormal sperm cell, indicating that the low dose of this extract is more toxic to the germ cells than high doses. Similarly, the extract of *C. papaya* leaf also showed toxicity at all the doses tested with the lowest dose inducing the highest abnormalities in sperm cells. However, the extract of the pulp of *C. papaya* showed that the extract is not toxic to the germ cell at a low dose, hence, the low dose is safer for consumption than high doses. These results showed that extracts of the leaf of *C. papaya* and stem bark of *B. ferruginea* are toxic to germ cells and hence, their use as medicinal plants should be discouraged. A similar result has been documented in a toxicological study where the aqueous extract of *B. ferruginea* stem bark caused lipid peroxidation and damaged sperm quality[27].

Alteration of the testes architecture is widely considered a biomarker of chemical toxicity and is acknowledged as the most sensitive endpoint for detecting testicular toxicity[28]. The data of the present study showed that the extracts of *B. ferruginea* (stem bark) and *C. papaya* (unripe pulp and leaves) induced various histopathological lesions in the testes of exposed mice such as sloughing of the germinal epithelium, interstitial cell proliferation, increased luminal width, necrosis of spermatogonia, seminiferous epithelium vacuolization and tubular deformation. The observed testicular damage in the mice in this study indicates the aqueous extracts of *C. papaya* (unripe pulp and leaves) and *B. ferruginea* (stem bark) are capable of crossing the blood-testis barrier, thus, providing a piece of direct evidence in support of the assertion that the aqueous extracts of *C. papaya* (unripe pulp and leaves) and *B. ferruginea* (stem bark) disrupt the process of spermatogenesis, causing an increase in the formation of abnormal sperm cells.

The evaluated biochemical parameters in this present study are very important indices for the determination of plant extract-induced toxicity and oxidative stress in animals to predict the possible effect in humans. The data revealed significant modulation of the activities of ALP, AST and ALT by the extract of *B. ferruginea* in mice. The activities of serum ALP, AST and ALT are used routinely to assess kidney and liver cell injury. Increased activities of these cytosolic enzymes in the serum although they are localized in the periportal hepatocytes is an indication of cellular membrane damage and leakage[29]. Therefore, their increased serum concentration might be a useful indicator of potential acute renal or hepatic damage and subsequent leakage into the bloodstream[30]. While acute exposure to these plant extracts may be harmful to the nervous system, chronic exposures may pose hepatic damage. The body can also repair the effect of subtoxic doses if sufficient time passes[31].

A possible mechanism for the production of abnormal sperm cells in mammals is oxidative stress, hence, we evaluated in the exposed mice the activities of two antioxidant enzymes. The SOD and CAT are among the important antioxidant enzymes which aid in the prevention of oxidative stress. The SOD is responsible for the dismutation of superoxide to hydrogen peroxide and CAT on the other hand is responsible for the destruction of the hydrogen peroxide to produce oxygen and water. The result of this study showed that the tested plant extracts caused reduction in the activities of SOD and CAT in the treated mice which suggests that the mice might be experiencing oxidative stress. The induction of oxidative stress in the mice could be a result of the effect of the phytochemicals present in the plants.

The measurement of the level of selected sex hormones in the mice exposed to the aqueous extracts of *C. papaya* (unripe pulp and leaves) and *B. ferruginea* (stem bark) showed that the aqueous extracts of *C. papaya* leave and *B. ferruginea* stem bark decreased the concentration of LH and FSH in the treated mice. Studies have reported an association between the circulating concentration of certain reproductive hormones and semen quality parameters[32,33]. In order to produce spermatozoids, the body requires the presence of LH, FSH and testosterone. Spermatogenesis and LH stimulate the binding of Sertoli cells to FSH and the production of testosterone in Leydig cells, respectively[24]. Results of the present report suggest that LH, FSH and TT play important roles in sperm morphology development, whereby decreased concentrations of LH and FSH, but an increased TT concentration caused a decreased number of normal sperms. The low concentrations of LH and FSH observed in the present study indicate androgen deficiency[34] since the two pituitary gonadotropins are important for spermatogenesis regulation. A low concentration of FSH can decrease the number of Sertoli cells considerably by 30-45% when compared to normal testicular development[35,36]. This is very important because the number of Sertoli cells is a determinant of the number of sperm cells that will be produced since a Sertoli cell can only support a specific maximum number of sperm cells[36,37]. Hence, the observed low concentration of FSH in the present study is an indication of low sperm production in the treated mice. The inability of the pituitary to secrete FSH and LH will disrupt

testicular function and cause infertility[38]. In Sertoli cells, testosterone facilitates local spermatogenesis by acting as a paracrine agent and also to provide feedback for LH secretion, thus, indicating that sperm morphological abnormality in this study is associated with disruptions and compensatory mechanisms in the hypothalamus-pituitary-gonadal axis[39].

The present study data showed that *C. papaya* and *B. ferruginea* contained a wide range of phytochemicals some of which might be responsible for the reproductive toxicity observed in exposed mice. Papaya contains a wide range of phytochemicals. The latex contains the enzyme papain, alkaloids are present in the leaves, phenolics in the shoots, fruits and leaves, carotenoids in the fruits and seeds and glucosinolates in the fruits and seeds. The phytochemical analysis of the leaves revealed that the leaves contained flavonoids, tannins, saponins and alkaloids including pseudocarpaine, dehydrocarpaines I and II and carpaine. Similarly, the stem bark of *B. ferruginea* was shown to contain alkaloids, cardiac glycosides, steroids, tannins and saponins. The alkaloids present in these plants have been reported to be teratogenic, inhibiting fetal movement and defects in the fetus when the aqueous extract of the leaves was administered to pregnant rats[40]. Also, saponin has been shown to have potential cytotoxic effects because of its ability to permeate the cells[22,41] which can alter the negatively charged carbohydrate portions on the cell surface and form complexes with cholesterol of the cell membrane causing pore formation[42]. These data implied what might possibly happen in humans exposed to these plant extracts. Continuous use of these extracts especially at high concentrations might lead to male infertility.

CONCLUSION

Aqueous extracts of *C. papaya* and *B. ferruginea* are reported to be highly therapeutic and locally accessible. They are most times consumed daily over a long period in Nigeria. Plant parts preparations are generally perceived by a larger percentage of the general public to be natural and as such would be free from side effects. These preparations that are most times used as medicine are usually self-administered. Hence, there is limited control of dose, method and rate of usage although the phytochemicals in medicinal plants may be natural and therapeutic, potential health risks and deleterious effects especially on germ cells cannot be ruled out. The assessment of the consumption or safety of use of these plants, therefore, is as important as the evaluation of their therapeutic potential. Therefore, the present study assessed the reproductive toxicity of the aqueous extracts of *B. ferruginea* (stem bark) and *C. papaya* (unripe pulp and leaves) in male mice using the sperm morphology assay. However, the findings of this study revealed that these extracts have adverse effects on the germ cells, especially on male reproductive cells even at low doses. Modulation of the activities of AST, ALT, ALP, SOD and CAT is believed to be responsible for this reproductive toxicity. It could therefore be recommended that public awareness of the potential reproductive toxicity of these plant extracts should be carried out to educate the populace who are regularly using them.

SIGNIFICANCE STATEMENT

The data of the present study is significant in that the aqueous extracts of *C. papaya* and *B. ferruginea* contain reproductive toxicants capable of altering the process of spermatogenesis in mammals and leading to increased production of abnormal sperm cells. The constituents of the extracts also modulated the activities of both biochemical and reproductive enzymes in the exposed animals.

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