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Adansonia digitata aqueous leaf extract ameliorates dexamethasone-induced testicular injury in male Wistar rats

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ABSTRACT

Objective: To evaluate the effects of aqueous leaf extract of *Adansonia (A.) digitata* L on dexamethasone-induced testicular damage in male Wistar rats.

Methods: Twenty adult male Wistar rats weighing 170-190 g were divided into four groups. Group Ⅰ received 0.5 mL of phosphate buffer orally for 28 days and served as the normal control group; group Ⅱ received 10 mg/kg of dexamethasone (a synthetic glucocorticoid) intraperitoneally for 7 days and 0.5 mL of phosphate buffer orally for 21 days, group Ⅲ received 10 mg/kg of dexamethasone for 7 days and 800 mg/kg of *A. digitata* extract orally for 21 days; group Ⅳ received 10 mg/kg of dexamethasone for 7 days and 300 mg/kg of vitamin-E orally for 21 days. Dexamethasone was administered intra-peritoneally for 7 days and all administration lasted for 28 days. The rats were sacrificed by anesthesia with diethyl ether and the testes of each animal were harvested. The testis was homogenized in 0.25 M sucrose at 4 ℃ for biochemical and histological analyses.

Results: Administration of dexamethasone significantly decreased body weight, glucose-6-phosphate dehydrogenase (G6PDH), lactate dehydrogenase (LDH), superoxide dismutase (SOD), and glutathione peroxidase (GPx) (P<0.05), and significantly increased malondialdehyde (MDA) activities (P<0.05). The degeneration in the population of spermatogonia and vacuolation and abnormal widening of the interstitial spaces were observed in the rats treated with dexamethasone. However, administration of *A. digitata* significantly increased SOD, GPx, G6PDH, and LDH levels, significantly decreased MDA activities and improved the histoarchitecture of the testis (P<0.05).

Conclusions: *A. digitata* may have an ameliorative effect on dexamethasone-induced testicular damage in Wistar rats because of its anti-inflammatory and antioxidant properties.

KEYWORDS: *Adansonia digitata* L; Dexamethasone; Vitamin E; Testis; Oxidative stress; Wistar rats

1. Introduction

Dexamethasone is a potent anti-inflammatory and immunosuppressive agent, and its administration results in a wide range of effects on inflammatory and immunologically-mediated disease processes[1]. Dexamethasone is synthetic glucocorticoids synthesized by the adrenal gland from the precursor sterol-cholesterol and has been widely used for *in-vitro* and *in-vivo* studies of the glucocorticoid effects on some different cellular and physiological responses[2-4].

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It has been reported that glucocorticoids may induce Leydig cell apoptosis while dexamethasone-mediated suppression of testosterone also caused adverse physiological consequences, including muscular atrophy and sexual dysfunction[2,5]. Several studies in humans have shown that elevated cortisol, the main circulating glucocorticoids, resulting from insulin-induced hypoglycemia decreases serum testosterone content, with no alteration in pituitary luteinizing hormone levels[6,7]. Dexamethasone treatment causes dysregulation of physiological processes, including spermatogenesis and with resultant male infertility via increasing production of reactive oxygen species (ROS)[8].

ROS is a group of highly reactive oxidizing agents that can damage almost all biomolecules in spermatozoa, including lipids, proteins, and nucleic acids, causing oxidative stress that damages the biological membranes in the testes[8,9]. Thus, ROS can disrupt cellular components to impair sperm structure and function[10,11]. Some reports have shown that anti-oxidant treatment reduces testicular derangement and protects the cellular components from oxidative injury[9,11]. Also, detoxifying lipid peroxidation products (glutathione S-transferases, phospholipid-hydroperoxide glutathione peroxidase, and ascorbate peroxidase), and a network of low molecular mass anti-oxidants (medicinal plants, flavonoid, vitamins, and phenolic compounds) have been reported in several studies to have demonstrated to be potent antioxidants in the prevention of dexamethasone-induced oxidative stress-related in rats by scavenging ROS[2,12].

Adansonia (A.) digitata L is also known as Baobab, Monkey-bread tree, Dead rat-tree, and Lemonade tree. It belongs to the Malvaceae family[13] and is a deciduous tree native to arid Central Africa. It grows naturally and widely distributed in most of Sub-Sahara Africa's semi-arid and sub-humid regions as well as in western Madagascar[14]. Baobab is a very long-lived tree with multipurpose uses. Different parts of the plant are widely used as foods, medicines, and the bark fibers are also used traditionally[14]. The tree provides food, shelter, clothing, and medicine as well as material for hunting and fishing[15]. In folklore medicine, it is used as an antipyretic, febrifuge, astringent in diarrhea and dysentery. Fruit pulp and powdered seeds are used in cases of dysentery and to promote perspiration[14,16].

Previous studies on phytochemical screenings have revealed the presence of various potentially bioactive ingredients, including triterpenoids, flavonoids, tannins, amino acids, vitamins, lipid, and carbohydrates[16,17]. It has been reported that the fruit pulp contains triterpenes, saponins, tannins, carbohydrates, and glycosides[18,19].

A. digitata has been reported to exert cardioprotective, hepatoprotective, anti-viral, anti-microbial, anti-trypanosoma, antidiabetic, and antioxidant effects[20–23]. The baobab fruit pulp has traditionally been used as an immunostimulant, in the treatment of diarrhea and dysentery, analgesic, antipyretic, febrifuge and it has also been found to exhibit anti-inflammatory properties[24–26]. However, the potential of this plant to protect against dexamethasone-induced testicular injury has not been scientifically validated. Thus, the present study was designed to investigate the effect of aqueous leaf extract of *A. digitata* on dexamethasone-induced testicular injury in male Wistar rats.

2. Materials and methods

2.1. Experimental animals and handling

Twenty adult male Wistar rats aged 6-8 months, with an average weight of 170-190 g, were procured from National Veterinary Research Institute Vom, Plateau, Nigeria. The rats were kept in the animal control room of Bingham University to acclimatize the environment for 2 weeks. The rats were fed on starter mash (vital feeds grand cereals Ltd. Jos, Nigeria) and water ad libitum and maintained under standard conditions. The animal room was well ventilated with a temperature range of (25-27) ℃ under day/night 12-12 hour photoperiodicity.

2.2. Collection of sample and Authentication

Fresh leaves of *A. digitata* L. were obtained from the Mararaba market in Mararaba, Nasarawa State, Nigeria. The plant sample was identified and authenticated by late Dr. D.O Otegbeye, in the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The voucher specimen was deposited in the herbarium with a voucher number (FHI: 113015).

2.2.1. Preparation of the extract

The collected samples were sliced into smaller pieces and sun-dried at room temperature, and then crushed to a powdered form finely and subjected to extraction. Then, 2 500 g of the powdered leaves were macerated in 25 000 mL of distilled water for 48 h. It was then filtered with No. 4 Whatman filter paper and the filtrate was concentrated to a semi-solid form using beakers and a water bath at 40℃ to 50℃. The filtrate was concentrated and dried in a rotary vacuum evaporator at a reduced pressure to obtain 410 g dry residue to yield a 16.4% volume. Further, 410 g of the dry residue was obtained and dissolved in phosphate buffer solution for dosage preparation. It has been reported that the LD_{50} of *A. digitata* was 1 525 mg/kg body (b.w.) weight and administered intraperitoneally (*i.p.*)[24]. Tokyo Chemical Industry Co., Ltd (TCI) America™ (2010), stated the rat LD_{50} of dexamethasone was 54 mg/kg b.w. (*i.p.*), 14 mg/kg b.w. (subcutaneously) and greater than 3 mg/kg b.w. (orally)[2]. The dosage formulation of the *A. digitata* extract was carried out and used was consistent with the previous investigation on the *A. digitata*[24]. The stock solution of the *A. digitata* extract had a concentration of 320 mg/mL.

2.2.2. The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in aqueous extracts of *A. digitata*[18]. The freshly prepared leaf extract of *A. digitata* was quantitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed for triterpenoids, terpenoids, flavonoids, saponin, tannins, alkaloids, amino acids, glycosides, steroid[19].

2.3. Experimental design

The 20 male Wistar rats were divided into four groups (n=5 in each group). Group Ⅰ received 0.5 mL of phosphate buffer orally for 28 days and served as the normal control group; group Ⅱ received 10 mg/kg of dexamethasone (a synthetic glucocorticoid) intraperitoneally for 7 days and then received 0.5 mL of phosphate buffer for 21 days; group Ⅲ received 10 mg/kg of dexamethasone for 7 days and subsequently received 800 mg/kg of *A. digitata* extract orally for 21 days; group Ⅳ received 10 mg/kg of dexamethasone for 7 days and subsequently received 300 mg/kg of vitamin E orally for 21 days.

2.4. Weight variation

The animals were weighed at the outset and then weighed every seven days from the treatment's commencement until the last day of the experimental period and the difference in weight from the initial weight per group was calculated.

2.5. Animal sacrifice

The animals were sacrificed 7 days after the experimental period, they were euthanized with diethyl ether anesthesia and the testes were excised following abdominal incision. Right testes were fixed in 10% Bouin's fluid for histological analysis, while left testes were homogenized in 5% sucrose solution for enzymes assay.

2.6. Experimental protocol

2.6.1. Routine histological preparation

The histology of the testes and epididymis were done by the method previously described[11]. The organs were cut into slabs of about 0.5 cm thick transversely and fixed in 10% buffered formalin for a day, after which it was transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and absolute alcohol and xylene for differentiations before they were transferred into two changes of molten paraffin wax for 1 h each in an oven at 65 ℃ for infiltration. They were subsequently embedded and serial sections were cut by using rotatory microtome at 6 μm. The tissues were transferred onto albuminized slides and allowed to dry on a hot plate for 2 min. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes), 70% alcohol, 50% alcohol, and then watered for 5 min. The slides were then stained with hematoxylin and eosin and mounted in Canada balsam. Photomicrographs were taken with the photomicroscope manufactured by Leica company in Wetzlar, Germany.

2.6.2. Enzyme histochemistry

Excised testicular tissues were put in Lao style mortar containing 1 mL of 0.25 Mn (5%) sucrose solution and were homogenized thoroughly. Tissue homogenates were collected in a 5 mL plain serum bottle for enzyme assay: glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD), and malondialdehyde (MDA).

2.6.2.1. Determination of G6PD activity in tissue homogenate

G6PD activity in the homogenate was measured according to the method previously described by Beydemir[27]. The homogenate was centrifuged at 4000 ×g for 10 min at 4 ℃. The clear supernatant obtained was used for the measurement of G6PD activity.

2.6.2.2. Determination of SOD activity in tissue homogenate

Enzyme activity of SOD was assayed according to the method of Alam *et al*[28] by using a reagent kit produced by Randox Lab Ltd.

2.6.2.3. Determination of MDA activity in tissue homogenate

MDA levels in tissue homogenates were measured according to the protocol outlined by Kakali *et al*[29], namely, 0.1mL of homogenate was pipetted into a plastic test tube and then 1 mL of 20% trichloroacetic was added to it. The mixture was mixed and centrifuged at 2000 ×g for 5 min. Subsequently, 0.50 mL of the supernatant was pipetted into a pyrex test tube, 0.05 mL of 10.0 μmol/L of 1,1,3,3-tetramethoxylpropane was pipette into another pyrex test tube (Standard), and 0.50 mL of trichloroacetic acid solution and 1.0 mL of thiobarbituric acid were pipetted into a 3rd pyrex test tube (blank). All tubes were stoppered tightly. The test tubes were heated in a water bath at 100 ℃ for 20 min. Then, all tubes were cooled in water. The spectrophotometer was blanked using the reagent blank at 532 nm. The absorbance of tests and standards were read and taken.

2.7. Biochemical procedures

2.7.1. Determination of lactate dehydrogenase (LDH) activity in tissue homogenate

LDH activity in the homogenate was measured according to the method of Jeyaraman *et al*[30]. The homogenate was centrifuged at

4000 ×*g* for 10 min at 4 ℃. The clear supernatant obtained was used for the measurement of LDH activity.

2.7.2. Determination of glutathione peroxidase (GPx) activity in tissue homogenate

GPx activity in the homogenate was determined according to the method previously described by Alam *et al*[28] using the reagent kit (Randox lab, Ardmore, Diamond road, Crumlin Co., UK BT294QY) according to the manufacturer's protocol.

2.8. Statistical analysis

All calculations were done by using the SPSS 20 statistical software package for analysis of the data. The data were presented as mean±standard deviation (mean±SD), and statistical analysis was carried out by using the analysis of variance. The values were considered significant when $P<0.05$.

2.9. Ethics approval

This study was sought and approved by the Bingham University Health Research and Ethics Committee (the ethical number: 2016-03-02-017). The use and care of the animals and the experimental protocol were in strict conformity with the guidelines of the committee.

3. Results

3.1. Phytochemical analyses

The phytochemical analysis revealed the presence of triterpenoids, terpenoids, flavonoids, saponin, tannins, alkaloids, amino acids, glycosides, steroid, carbohydrate, lipid, and protein.

3.2. Effect of A. digitata on body weight of male rats

Dexamethasone-treated rats (group Ⅱ) showed a significant decrease in body weight as compared with the normal control rats ($P<0.05$). The normal control group (group Ⅰ) showed their mean body weight increasing by 10.8 g while the rats in group Ⅱ lost an average of -26.4 g after 4 weeks of induction. Dexamethasone treated with *A. digitata* (group Ⅲ) showed a significant increase in body weight compared to group Ⅱ ($P<0.05$) and a similar result was observed in the dexamethasone rats treated with vitamin E (Table 1)

3.3. Effect of A. digitata on G6PDH and LDH

Induction of dexamethasone (group Ⅱ) significantly decreased G6PDH and LDH levels as compared with thenormal control group ($P<0.05$) (Table 2). However, treatment of rats with dexamethasone with the *A. digitata* extract modulated their reduction. Also, there was a significant difference in G6PDH and LDH levels between the normal control group and the group of dexamethasone-treated rats with the *A. digitata* extract alone (group Ⅲ) ($P<0.05$),although a significant decrease of G6PDH and LDH levels was observed in the group co-administered with dexamethasone and vitamin E (group Ⅳ) ($P<0.05$).

3.4. Effect of A. digitata on SOD, GPx, and MDA activities

Dexamethasone treatment caused a significant ($P<0.05$) decrease in levels of SOD, GPx, and a significant ($P<0.05$) increase in MDA activity in the testes compared to the normal control group ($P<0.05$). However, a significant ($P<0.05$) increase in the activities of these enzymatic antioxidants SOD and GPx while a significant ($P<0.05$) decrease in non-enzymatic parameter (MDA) were observed in the group co-administered with both dexamethasone and *A. digitata* extract (Table 3).

Table 1. Effect of *Adansonia digitata* on body weight of male rats (g).

Body weight	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Initial body weight	169.4±6.2	178.1±9.3	173.5±4.7	175.7±6.8
Final body weight	180.2±8.8	151.7±9.1[a]	193.3±8.2[b]	191.9±7.2[b]

Note: Values are expressed as mean±SD (n=5 in each group). [a]$P<0.05$ *vs* group Ⅰ; [b]$P<0.05$ *vs* group Ⅱ. Group Ⅰ received 0.5 mL of phosphate buffer for 28 days and served as the normal control group; group Ⅱ received 10 mg/kg of dexamethasone for 7 days and then received 0.5 mL of phosphate buffer for 21 days; group Ⅲ received 10 mg/kg of dexamethasone for 7 days and subsequently received 800 mg/kg of *Adansonia digitata* extract orally for 21 days; group Ⅳ received 10 mg/kg of dexamethasone for 7 days and subsequently received 300 mg/kg of vitamin E orally for 21 days.

Table 2. Effect of *Adansonia digitata* on glucose-6-phosphate dehydrogenase and lactate dehydrogenase (IU/L).

Parameters	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
G6PDH	5625.0±28.4	4727.3±36.7[a]	5365.7±34.4[b]	5323.3±37.1[b]
LDH	3634.7±34.7	3041.7±36.0[a]	4120.0±28.2[b]	4040.0±20.5[b]

Note: Values are expressed as mean±SD (n=5 in each group). [a]$P<0.05$ *vs* group Ⅰ; [b]$P<0.05$ *vs* group Ⅱ. G6PDH: glucose-6-phosphate dehydrogenase; LDH: lactate dehydrogenase.

Table 3. Effect of *Adansonia digitata* on SOD, GPx, and MDA activities.

Parameters	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
SOD (IU/mL)	611.7±14.2	568.7±8.6	635.4±4.6	608.0±4.3
GPx (nmol/mg)	657.0±6.0	607.0±9.0[a]	711.7±7.3[b]	653.0±3.1[b]
MDA (nmol/mg)	24.7±3.4	28.0±2.6[a]	23.2±1.8[b]	21.0±1.5[b]

Note: Values are expressed as mean±SD (n=5 in each group). [a]$P<0.05$ *vs* group Ⅰ; [b]$P<0.05$ *vs* group Ⅱ. SOD: superoxide dismutase; GPx: glutathione peroxidase; MDA: malondialdehyde.

3.5. Histological interpretations

Compared the different stages in seminiferous elements with the presence of the spermatogonia cells, the normal leydig cells were increased in numbers with the stratification of germ layer cells, and the normal sertoli cells were observed in the normal group (Figure 1A). The testicular histology of the dexamethasone group showed seminiferous lumen was distorted, the mild atrophy of leydig cells was reduced in numbers, and sertoli cells were vacuolized with the stratification of germ layer cells (Figure 1B). Normal histological arrangement of the testes and the spermatogonia cells was prominent at different stage of development with regeneration in their germinal epithelium, and the normal seminiferous lumen were observed in the dexamethasone-treated with the aqueous extract of 800 mg/kg of *A. digitata* group (Figure 1C). The exerted few configurations of germ cells in the seminiferous tubules some germ cells, with narrow interstitial cells and the moderate seminiferous lumen were observed in the dexamethasone treated with the vitamin E group (Figure 1D).

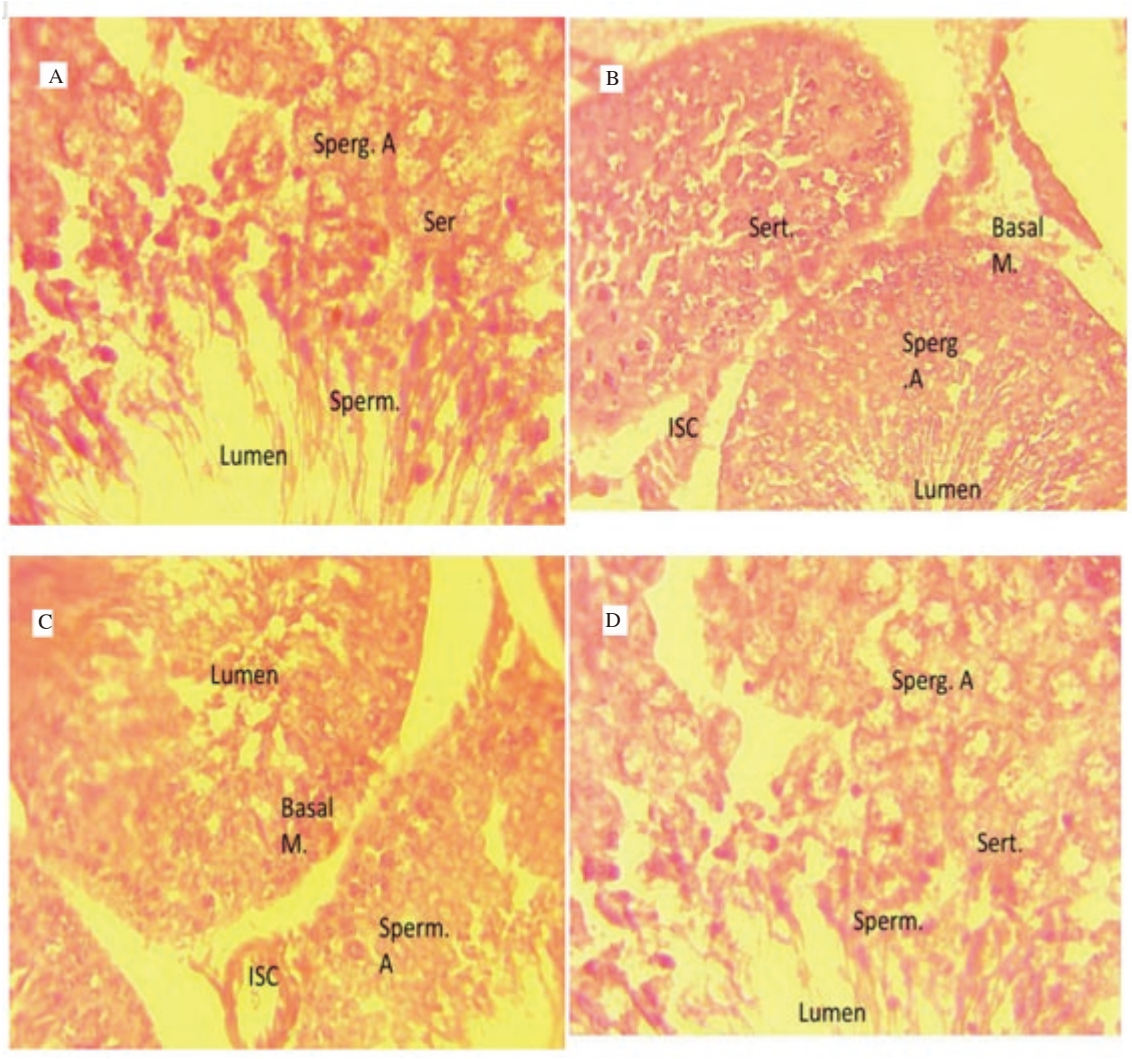


Figure 1. The photomicrograph of the testis tissue of rats in different groups (H & E stain; magnification ×400). A: The normal control group shows different stages in seminiferous elements comprising spermatogonia cells (Sperg. A), Leydig cells, Sertoli (Sert), and interstitial cells (ISC) which are normal in appearance. B: The untreated dexamethasone group shows that spermatogonia cells (Sperg. A) are densely populated than the sparse differentiating cells (Sertoli and interstitial cells) with testicular atrophy. C: The dexamethasone plus the aqueous extract of 800 mg/kg of *A. digitata* group reveals the normal histological arrangement of the testes; the spermatogonia (Sperg A) are prominent at different stage of development, and the differentiating cell, the supporting cell (ISC) and Sertoli cells (Sert) are revealed in details. D: The dexamethasone plus the vitamin E group shows few configurations of spermatogonia (Sperg A), with moderate lumen of seminiferous tubule (Lumen) containing moderate spermatogenesis (Sperm) along with the Sertoli (Sert).

4. Discussion

In the present study, G6PDH, LDH, SOD, GPx levels, and body weight gain were significantly decreased, while MDA level was significantly increased after injection of dexamethasone to the male rats. Oral administration of aqueous extract of *A. digitata* extract to the dexamethasone-treated male rats significantly increased average body weight, G6PDH, LDH, SOD, and GPx levels, other than a decrease in MDA level as compared with the dexamethasone only group. It was demonstrated that a drastic increase in body weight was due to its intervention roles, which was in accordance with the report of Chitra and Mathur[31].

It was observed that dexamethasone caused a significant decrease in G6PDH activity when compared to the normal control group. This is in accordance with the report of Chitra and Mathur[31]. However, a significant increase in G6PDH level was observed in the group of the dexamethasone-treated rats with the *A. digitata* leaf extract as compared with only dexamethasone treated group(group Ⅱ). This implies that *A. digitata* leaf extract causes an increase in G6PDH activity, thereby possessing anti-oxidant characteristics. *A. digitata* leaf extract has been shown to possess the following phytochemical constituents, potentially bioactive ingredients, including triterpenoids, flavonoids, tannins, amino acids, vitamins, lipid, and carbohydrates[18,19]. Flavonoids, tannins, saponins, and terpenoids have been implicated to have known to be potentially bioactive ingredients against oxidative stress-induced toxicity rats[16,18].

According to the report of Beydemir[27], dexamethasone inhibits G6PDH activity, thereby exposing the cell to oxidative damage. LDH levels were also seen to be decreased significantly in group Ⅱ (10 mg of dexamethasone only) as compared with the normal control group. Group Ⅲ, which received the same concentration of dexamethasone as group II but was treated with *A. digitata*, had significantly higher LDH activity than that of group IV; this is in agreement with the study conducted by Zhang *et al*[32].

G6PDH is a key enzyme that catalyzes the oxidation of D-glucose 6-phosphate to D-glucose-O-lactone 6-phosphate in the presence of nicotinamide adenine dinucleotide phosphate ($NADP^+$). It is well known that the dehydrogenase exists in the oxidative part of the pentose phosphate metabolic pathway. The significant role of the pathway is to produce NADPH[33,34], which is used for the protection of cells against oxidative damage[32,34]. Also, NADPH is widely used in biosynthesis and in drug detoxication (Many enzymes play an important role in maintaining the integrity of the lens metabolism)[33]. The pentose phosphate metabolic pathway functions in the lens to generate reduced equivalents (NADPH), which are used mainly for the maintenance of reduced glutathione (GPx) levels[33].

Determination of SOD, GPx, lipid peroxidation, and other anti-oxidant enzyme activities in biological membranes and tissues has always been used as reliable markers for tissue damage and oxidative stress[28]. In this present study, testicular oxidative damage by the induction of dexamethasone is also exhibited by a significant decrease in the activities of antioxidant enzymes SOD, GPx, and a significant increase of testicular content of MDA as compared with the normal control group. This, in turn, may cause the degeneration of the seminiferous tubules along with the spermatogonia were densely populated than the sparse differentiating cells with testicular atrophy. With the intervention role of the *A. digitata*, the histological integrity of the testis rats treated with dexamethasone plus the aqueous extract of *A. digitata* were restored with the normal histological arrangement of the testes; the spermatogonia were prominent at different stage of development, and the differentiating cell, the supporting cell, and sertoli cells were revealed in details. This suggests that the treatment with the *A. digitata* markedly modulates the oxidative damage in dexamethasone-induced rats. Therefore, it is possible to suggest that this *A. digitata* extract might confer protection against dexamethasone-induced testicular damage as evidenced by normal levels of anti-oxidant enzymes in the *A. digitata* treated groups due to the presence of polyphenols compound flavonoids, triterpenoid, and tannins in the phytochemical constituents of *A. digitata*[2,13].

SOD is a natural scavenger of reactive oxygen species and superoxide anion radicals[38]. In the anti-oxidant enzymes, SOD is the first enzyme to combine with active oxygen free radicals. SOD specifically combines with superoxide anions and acts synergistically with GPx to prevent lipid peroxidation in the cell membrane and damaging metabolites' formation[32]. They directly capture and eliminate free radicals[26]. SOD suppresses cell membrane lipid peroxidation during pathological lesions, protects biological macromolecules and membranes from damage.

GPx converts hydrogen peroxide (H_2O_2) into H_2O. GPx has a strong ability to scavenge lipid peroxide and hydrogen peroxide which were induced by active oxygen species and hydroxyl radicals. Thus, SOD and GPx can protect biological macromolecules and membranes from damages[28]. Considering the SOD activity, it is observed that group Ⅱ (dexamethasone only) had a lower SOD level when compared to group Ⅲ (dexamethasone+*A. digitata*). These results confirm the study conducted by Mukhar[26]. Comparing the *A. digitata* only groups to the dexamethasone group, a significant increase in the activity of SOD is observed, thereby suggesting that *A. digitata* plays a role in preventing SOD inhibition, thus preventing oxidative stress.

Lipid peroxidation causes secondary damage to cell functions, genotoxicity, and carcinogenesis[36]. According to the report of Kakali *et al*[29], dexamethasone increased MDA content, thereby increasing the level of reduced glutathione significantly in the serum. There was a reduced MDA activity in group Ⅲ (receiving dexamethasone and *A. digitata*) and group Ⅳ (receiving dexamethasone and vitamin E) when compared to group Ⅱ (receiving dexamethasone only).

In conclusion, the results of this investigation have demonstrated that dexamethasone (a synthetic glucocorticoid) in excessive

quantity can lead to oxidative damage of testicular cells and in turn, *A. digitata* extract mitigated reproductive toxicity associated with dexamethasone via modulation of enzymatic alterations and reduction in oxidative damage in male Wistar rats. The protective effect exhibited by *A. digitata* can be attributed to its antioxidant properties. However, further studies are required to validate the mechanism through which *A. digitata* extract mediated the protective activity against dexamethasone-induced reproductive damage in male Wistar rats.

Conflict of interest statement

The authors declare that there is no competing interest.

Authors' contributions

This work was carried out in collaboration between all authors. Author Joseph Babatunde Dare designed the study and performed the statistical analysis. He equally wrote the first draft of the manuscript and undertook the final editing of the paper. Author John Adakole Adole wrote the protocol, part of the draft, and embarked on the paper's initial editing. Authors Abdulfatai Olakunle Ojewale and Olugbemi Tope Olaniyan carried out most of the literature searches while authors John Adakole Adole and Gloria Enevwo Okotie contributed in terms of performing the experiment, handling the tissue processing for histology, and authors Joseph Babatunde Dare and Abdulfatai Olakunle Ojewale performed the histological analysis. Christopher Oloruntoba Akintayo carried out literature searches and undertook the final editing of the paper. Ayobami Dare also undertook the final editing of the paper. All authors read and approved the final manuscript.

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