Ameliorative effects of aqueous extracts of ginger and garlic on *Hibiscus sabdariffa*-induced testicular damage in male Wistar rats

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Context
Hibiscus sabdariffa L. (Roselle) is an annual shrub used widely for medicinal and nutritional purposes.
Aim
To evaluate the ameliorative effects of ginger and garlic on *H. sabdariffa*-induced testicular damage in male Wistar rats.
Settings and design
Reproductive toxicity of *H. sabdariffa* at high dosage and the ameliorative potential of ginger and garlic were determined.
Materials and methods
Twenty male Wistar rats were grouped into one control group and three experimental groups of five rats each. The animals in group 1 received 1 ml distilled water, the animals in group 2 received 250 mg/kg of *H. sabdariffa*, and the animals in group 3 received 250 mg/kg of *H. sabdariffa* and were cotreated with ginger and garlic (250 mg/kg), respectively, for 28 days. The animals in group 4 received 250 mg/kg of *H. sabdariffa* for 14 days and were left to recover naturally for 14 days. The reproductive functional parameters were subsequently determined.
Results and conclusion
H. sabdariffa treatment significantly reduced ($P \leq 0.05$) the plasma levels of reproductive hormones testosterone, estradiol, prolactin, and luteinizing hormone, and follicle-stimulating hormone and compared with the control group. Histopathological examination of the testes also showed marked degeneration of the seminiferous tubules, with necrosis and alteration in testicular structure in *H. sabdariffa*-treated rats. These alterations induced by *H. sabdariffa* were significantly ($P \leq 0.05$) improved by treatment with ginger and garlic. The results indicated that ginger and garlic protect against testicular damage induced by *H. sabdariffa,* which may be because of their antioxidant properties and as such may be useful in treating *H. sabdariffa*-induced testicular damage.

Keywords:
garlic, ginger, *Hibiscus sabdariffa*, reproductive hormones, testicular damage

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Introduction

Through empirical discovery, humans have continually identified plants that yield beneficial health effects. Medicinal plants contain physiologically active principles that have been exploited in traditional medicine for the treatment of various ailments over the years [1]. Phytochemistry of medicinal plants has unveiled the chemicals that are present in plants and these include tannins, saponins, terpenes, flavonoids, and cardiac glycosides [2]. Structural analysis of these phytochemicals has provided the basis for their therapeutic potency such as antioxidant, anti-inflammatory, anticancer, antiobesity, antiviral, anti-carcinogenic, and antimicrobial activities [3].

Hibiscus sabdariffa is one of the plants that have been studied extensively in the last two decades. It is used in the treatment of common cold; it prevents constipation, maintains healthy teeth and gums, and aids healthy pregnancy [4]. Phytochemical screening and structural analyses have identified anthocyanin, a class of flavonoids responsible for its cardioprotective and antioxidant properties [5–7]. Anticancer, anti-inflammatory, and hepatoprotective properties of *H. sabdariffa* have been reported [8–10]. However, subchronic studies of *H. sabdariffa* by Orisakwe *et al.* [11], have shown that aqueous extracts of *H. sabdarifa* significantly decrease epididymal sperm counts, histological distortions of

tubules, disruption of normal testicular epithelial organization, and disintegration of sperm cells.

Ginger (*Zingiber officinale*) and garlic (*Allium sativum*) are plants used traditionally as spices in food preparation; they have been shown to have both antioxidant and antimicrobial activities [12,13]. This study was carried out to determine the extent to which an aqueous extract of *H. sabdariffa* alters the plasma levels of selected reproductive hormones: prolactin (PRL), follicle-stimulating hormone (FSH), testosterone, luteinizing hormone (LH), and estradiol, and the ameliorative effects of aqueous extracts of ginger and garlic on these hormones in male Wistar rat as well as the histological features of testes in these experimental rats.

Materials and methods

Plant materials

Dried calyces of *H. sabdariffa*, ginger rhizomes, and garlic bulbs were obtained from a local market in Benin City, Edo State, Nigeria, and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Edo State, Nigeria.

Preparation of whole extracts of *H. sabdariffa* calyx

Dried calyces of *H. sabdariffa* were cleaned manually by handpicking stones and other debris. The samples were then thoroughly washed separately with deionized water and allowed to dry under the sun (40°C). 200 g of clean calyces of *H. sabdariffa* were added to 500 ml hot boiling water and left to stand for 15 min. The hot, red-colored aqueous extract was filtered with muslin cloth into a sterile plastic bowl and tightly covered. The aqueous extract of *H. sabdariffa* called zobo drink was cooled and packaged into sterile bottles and refrigerated at 5°C.

Preparation of garlic and ginger extracts

50 g of each of the spices (garlic and ginger) were chopped separately into small pieces with a clean stainless-steel knife. The chopped spices were then blended into 50 ml deionized water with a kenwood blender until smooth pastes were obtained. The pastes were diluted with 100 ml deionized water and filtered using a clean muslin cloth. The resulting extracts were added to the already prepared zobo drink at a concentration of 10%. The zobo drink blended with ginger and garlic was stored in a clean bottle and refrigerated at 5°C.

Experimental animals

A total of 20 male Wistar rats, 7–8 weeks old (weighing 0.25±0.02 kg), were purchased from a local breeder in Benin City, Edo State. They were housed in a clean, wooden-framed, wire mesh cages (150×40×20 cm). They were kept under a 14 : 10 light/dark cycle. Also, they were kept for four weeks for acclimatization and harmonization of their hormonal rhythm before commencement of the experiment under standard environmental conditions.

Experimental design

Experimental rats were divided into four groups of five animals each. Group 1 was the control and received 1 ml of distilled water, group 2 received 250 mg/kg of *H. sabdariffa* extract, and group 3 received 250 mg/kg of *H. sabdariffa* extract mixed with ginger and garlic extract through a gavage daily for 28 days, whereas the animals in group 4 received 250 mg/kg of *H. sabdariffa* for only 14 days and were allowed to recover naturally for another 14 days. The dose used was a modification of that used by Aline *et al.* [14].

Appropriate amount of the extract was administered to each rat twice daily (09:00 and 16:00 h) for a period of 28 days (except for group 1, which received only water, and group 4, which received the extract for 14 days). They were provided with feed and water ad-libitum throughout the period of the experiment.

Sample preparation for assay

At the end of the experiment (after 28 days), the animals were fasted overnight and killed by cervical dislocation under chloroform anesthesia. Blood samples were collected immediately by cardiac puncture from each rat. Each sample was transferred to EDTA bottles and the plasma samples were stored at −20°C for subsequent analysis. After the rats were killed, the testes were collected and immediately fixed in Bouins fluid for 6 h and transferred to 70% alcohol for histological processing according to Drunny and Wallington [15].

Biochemical assays

Assays for reproductive hormones

Estimation of circulating FSH was carried out using the rat FSH kit (cat no.: KT-15332), while LH estimation was carried out using the rat LH kit (cat no.: KT-21064).

Estimation of circulating estradiol was carried out using the rat estradiol (E2) kit (cat no.: KT-59814).

Estimation of circulating testosterone was carried out using the rat testosterone kit (cat no.: 55-TESMS-E01).

Estimation of circulating PRL was carried out using the PRL kit (cat no.: ISO-13485). All these were carried out using standard methods and procedures as detailed in the manufacturer's handout.

Results

Effect of aqueous extract of *H. sabdariffa* and *H. sabdariffa* mixed with ginger and garlic extracts on the levels of circulating reproductive hormones in rats

From the results obtained in the table, it could be concluded that administration of 250 mg/kg of *H. sabdariffa* significantly reduced the circulating levels of FSH, PRL, testosterone, LH, and estradiol of animals in test groups 2, 3, and 4 compared with the control group 1. However, cotreatment of the animals in group 3 with 250 mg/kg of ginger and garlic significantly increased the levels of these hormones and brought them closer to those observed for the control group 1 and higher than the levels observed in the animals in group 4 that were left to recover naturally for 14 days.

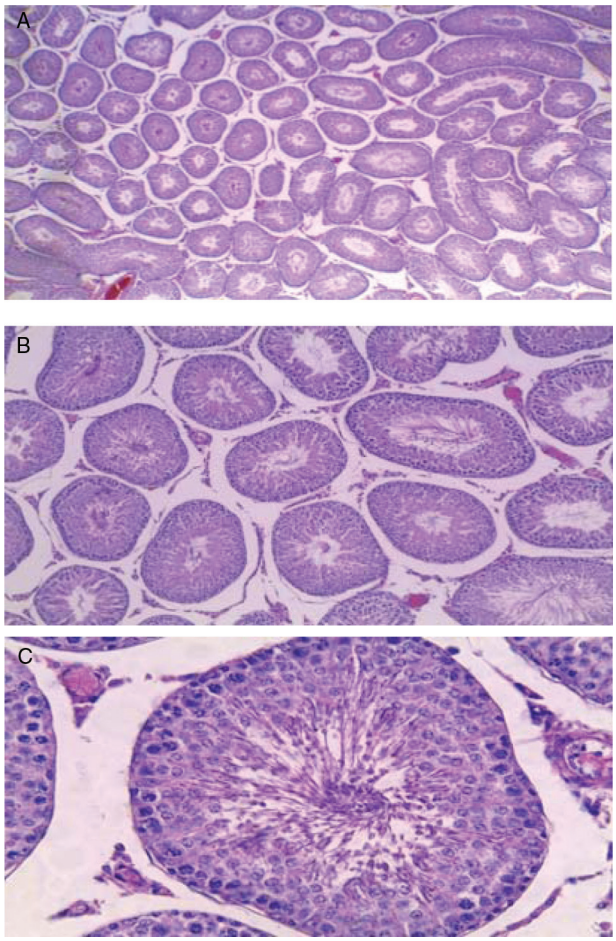
Effects of *H. sabdariffa* calyx on rat gonadal histology

The results of administration of *H. sabdariffa* calyx on gonadal development and histology of the various groups were discussed accordingly in the various groups as shown below. Relative to the control, there were significant changes in seminiferous tubules, basement membranes, and germ cells (Figs. 1–4).

Statistical analysis

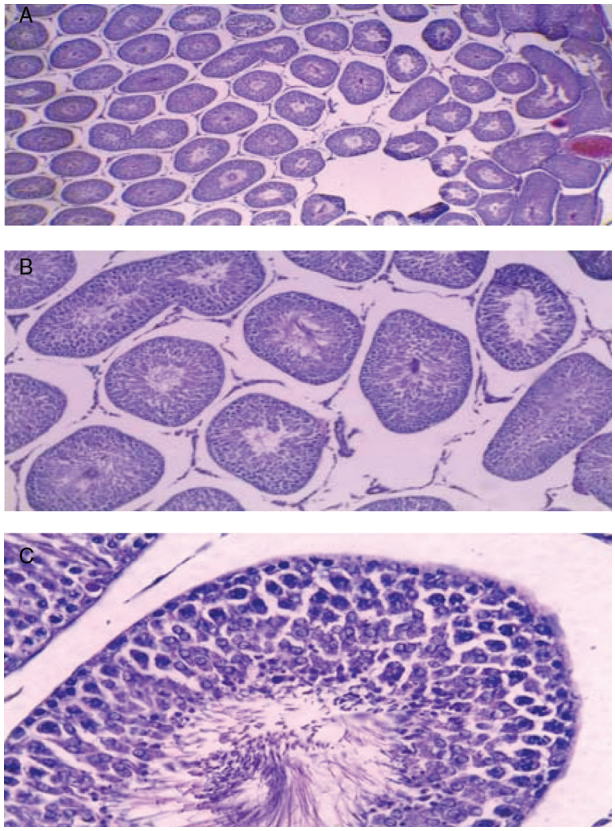
Statistical analysis was carried out using GraphPad prism (version 5) (Graphpad Software Inc., CA, USA) and all data were expressed as mean±SD. Comparisons within and between groups were made

Fig. 1



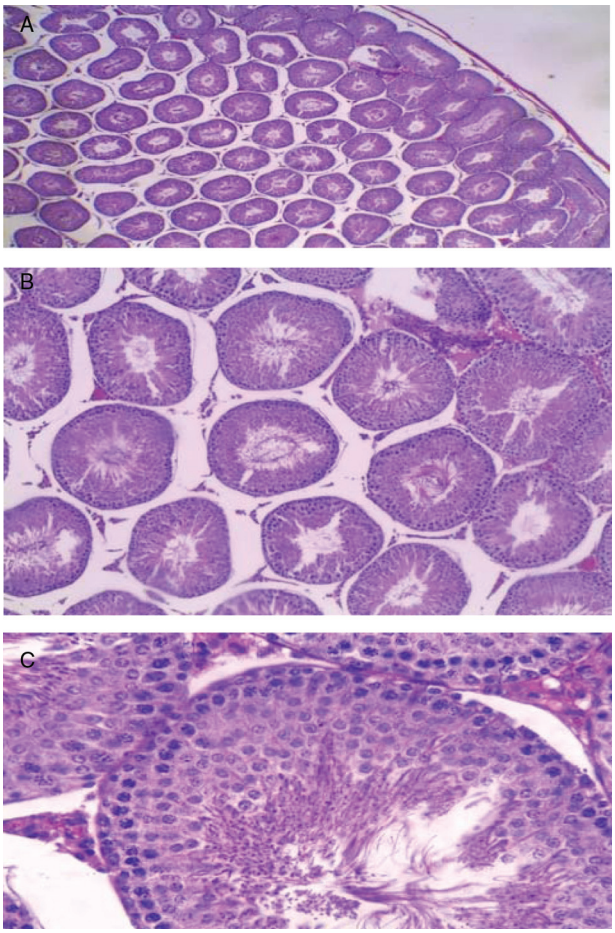
(a–c) Histopathological results of the testes for the animals in group 1 (control). (a) Closely packed seminiferous tubules in the testes (H&E, ×40). (b) Normal spermatogenesis and tubules in the testes (H&E, ×100). (c) Demonstrable spermatogenic series in the testes (H&E, ×400). Sections of (a–c) show numerous closely packed seminiferous tubules bounded by a thin basement membrane and separated by a fibrovascularstroma. These tubules contained numerous germ cells, developing to the lumen at different stages of maturation (demonstrable spermatogenic series) and interspersed by sertoli cells. The intervening fibrovascular stroma contained several interstitial cells of Leydig. Impression: normal spermatogenesis and tubules.

Fig. 2



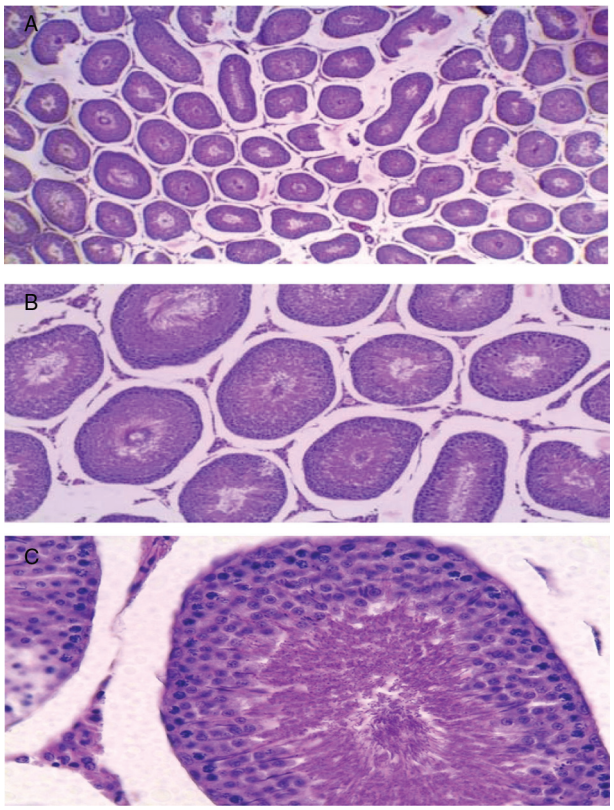
(a–c) Histopathological results of the testes for the animals in group 2 (250 mg/kg *Hibiscus sabdariffa*). (a) Loosely packed seminiferous tubules in the testes (H&E, ×40). (b) Germ cell series and arrested development of the spermatozoa (H&E, ×100). (c) Loss of demonstrable spermatogenic series in the testes (H&E, ×400). Sections of (a–c) showed a marked reduction in the seminiferous tubules that are loosely packed, bounded by a thick basement membrane, and showed loss of germ cell series and arrested development at the spermatogonium stage (no demonstrable spermatogenic series). The interstitium showed evidence of replacement fibrosis and prominence of Leydig cells. This indicates the absence of spermatozoa and spermatids and the presence of few spermatogonium.

Fig. 3



(a–c) Histopathological results of the testes for group 3 (250 mg/kg *Hibiscus sabdariffa* + 250 mg garlic and ginger). (a) Closely packed seminiferous tubules of the testes (H&E, ×40). (b) Good recovery of the testes (H&E, ×100). (c) Demonstrable spermatogenic series in the testes (H&E, ×400). Sections (a–c) showed numerous closely packed seminiferous tubules bounded by a thin basement membrane and separated by a fibrovascularstroma were observed. These tubules showed increased number of spermatogonia and preserved demonstrable spermatogenic series in more than half of the population of the somniferous tubules, indicating almost complete spermatogenesis and tubules.

Fig. 4



(a–c) Histopathological results of the testes for the animals in group 4 (recovery). (a) Loosely packed seminiferous tubules in the testes (H&E, ×40). (b) Incomplete recovery of the testes (H&E, ×100). (c) Spermatogenic arrest predominantly at the level of spermatogonium in the testes (H&E, ×400). (a–c) show a reduction/loss of seminiferous tubules bounded by a thin basement membrane and separated by a loose fibrovascularstroma with prominent Leydig cells. Majority of the seminiferous tubules show spermatogenic arrest predominantly at the level of spermatogonium, with only a few tubules showing demonstrable spermatogenic series up to the spermatocytic level. No spermatozoa but many spermatids that are not fully matured are present. This shows that the recovery did not fully restore the levels of spermatozoa.

using one-way analysis of variance. Differences were considered significant at P value of up to 0.05.

Discussion

H. sabdariffa is widely linked with reproductive toxicity in men and has been reported to have antifertility activity [16]. Oriasakwe *et al.* [11] reported that the subchronic administration of *H. sabdariffa* calyx to male Wistar rats resulted in reduced sperm count, motility, fertility, and viability, as well as increased amount of abnormal sperm. It has been suggested that the extract causes androgen depletion at the target cell level, particularly in the caudal epididymis, thereby affecting the physiological maturation of sperm [16]. In this study, the aqueous extract of *H. sabdariffa* calyx at a dose of 250 mg/kg caused a significant decrease ($P \leq 0.05$) in the plasma levels of estradiol, PRL, FSH, LH, and testosterone in male Wistar rats, compared with those of the distilled water control group (Table 1). However, the intragastric administration of *H. sabdariffa* calyx mixed with ginger and garlic (group 3) (250 mg/kg) for 28 days significantly increased ($P \leq 0.05$) the plasma levels of the reproductive hormones compared with those of the *H. sabdariffa*-treated group (Table 1). Furthermore, histopathological findings confirmed that *H. sabdariffa*-treated rats showed degeneration of seminiferous tubules and defoliation of spermatocytes in the slides. Importantly, garlic and ginger supplementation completely ameliorated the testicular damage induced by *H. sabdariffa* in the rats. This study showed that 28 days of oral administration of *H. sabdariffa* at 250 mg/kg resulted in a significant

Table 1 Effects of aqueous extract of *H. sabdariffa L.* and *H. sabdariffa L.* mixed with ginger and garlic extracts on the levels of circulating reproductive hormones in rats

Groups	Follicle-stimulating hormone	Prolactin	Testosterone	Luteinizing hormone	Estradiol
Group 1 (distilled water)	45±0.707	26±1.414	1.5±1.581	30±2.702	5±1.000
Group 2 (250 mg/kg *H. sabdariffa*)	36±0.707	21±1.643	1.0±1.414	24±1.702	3±0.921
Group 3 (250 mg/kg *H. sabdariffa* +250 mg/kg ginger and garlic)	40±1.000	23±2.024	2.6±0.322	27±1.623	5±0.100
Group 4 (recovery)	37.5±0.500	22±1.680	1.5±0.158	25±1.843	4±0.415

Values are presented as mean±SEM; Serum follicle-stimulating hormone, prolactin, testosterone, luteinizing hormone, and estradiol levels (μ/l) in male rats (n=5) after 28 days of oral administration of aqueous extract of calyx and water; Results in Table shows that the administration of 250 mg/kg of *H. sabdariffa* significantly reduced ($P \leq 0.05$) the levels of the circulating reproductive hormones (follicle-stimulating hormone, prolactin, testosterone, luteinizing hormone, and estradiol) compared with the control group 1. However, administration of 250 mg/kg of *H. sabdariffa* mixed with 250 mg/kg garlic and ginger significantly ($P \leq 0.05$) reversed the levels of these hormones and brought it to a level closer to that recorded for the animals in group 1. The reversal observed in the recovery group 4 (recovery) is not as profound as in the animals in group 3; H. sabdariffa, Hibiscus sabdariffa.

decrease ($P \leq 0.05$) in the plasma level of circulating reproductive hormones (FSH, LH, estradiol, PRL, and testosterone) and a marked reduction in sperm progressive motility; the spermatozoa of the rats were also adversely affected, thereby resulting in low sperm counts in all the *H. sabdariffa*-treated groups, which, by extension, may lead to infertility compared with the distilled water control group.

H. sabdariffa consumption also produces a significant ($P \leq 0.05$) decrease in the percentage sperm motility [17] and normal sperm morphology in human and animal spermatozoa [11]. Fakeye *et al.* [18] reported histological abnormalities in testicular tissue of *H. sabdariffa*-treated rats. These included intense intercellular spaces, irregular diameter of the seminiferous tubules, and high amounts of necrotic cells in the lumen compared with the controls. In addition, they showed that the epididymal sperm motility was decreased in *H. sabdariffa*-treated rats. All the above-mentioned observations were confirmed in the present study.

Peroxidation of polyunsaturated lipids in testis may produce structural alterations in biological membranes as well as changes in membrane stability and function. Moreover, lipid peroxidation is believed to be responsible, at least in part, for testicular damage because of *H. sabdariffa* intake. The possibility that administration of aqueous extract of *H. sabdariffa* may cause testicular lipid peroxidation has been reported experimentally [17] and was confirmed in this study. Thus, an aqueous extract of *H. sabdariffa* is an established testicular toxin and its excessive intake may lead to both reproductive and endocrine failure. In this study, decreased levels of reproductive hormone as well as loss of spermatocytes in *H. sabdariffa*-treated rats were observed. The decreased hormonal levels could be because of the phytoestrogens that are present in *H. sabdariffa* [17]. The reduced plasma level of reproductive hormones in male rats produced by the aqueous extract of *H. sabdariffa* calyx may be explained by the estrogenic activity of the plant, as postulated by Orisakwe *et al.* [11]. Furthermore, other studies had reported a statistically significant ($P \leq 0.05$) decrease in hormonal levels in laboratory animals treated with phytoestrogens [19,20].

In the present study, we found that supplementation with ginger and garlic significantly decreased the testicular lipid peroxidation and led to a marked increase in plasma reproductive hormones. Furthermore, administration of ginger and garlic to *H. sabdariffa*-treated rats improved the histomorphology of the testis. The histological alterations in testis of the treated rats were corroborated by changes in the levels of the circulating reproductive hormones. The observed ameliorative effect of ginger and garlic in this study may be because of its antioxidant properties, which may be involved in the scavenging of free radical species generated by *H. sabdariffa*. From these findings, it can be inferred that ginger and garlic positively modulate the antioxidant status and regenerate the testis of *H. sabdariffa*-induced rats to near normal.

Conclusion

This study has shown that administration of an aqueous extract of *H. sabdariffa* at 250 mg/kg caused endocrine disruption, indicated by low levels of FSH, LH, testosterone, PRL, and estradiol concentrations, as well as a marked degeneration of the seminiferous tubules and defoliation of spermatocytes. The improvement in the concentration of reproductive hormones and testicular damage following treatment with garlic and ginger may have been partly because of its antioxidant and ascorbic acid-restoration activities. Therefore, ginger and garlic protect the testes against functional impairment because of *H. sabdariffa* treatment and as such may be useful in treating *H. sabdariffa*-induced testicular damage.

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Conflicts of interest
There are no conflicts of interest.

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