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Protective Effects of Aqueous Extract of Ginger on Castor-oil - Induced Testicular Damage in Wistar Rats

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Abstract
The study was carried out to investigate the effects of ginger on castor oil-induced testicular damage in Wistar rats. Twenty adult male rats weighing 200-300 g were divided into four groups of five rats per group. The control group A was given the volume equivalent of 2.5 % tween 80 as that used as emulsifier in group B to administer 200 mg/kg of castor oil. Group C received 200 mg/kg castor oil and 250 mg/kg of ginger. Group D received 250 mg/kg of ginger only. The extract was administered orally for ten weeks. The initial and final body weight and the testicular weight of the animals were measured. The rats were thereafter sacrificed and the testes processed histologically.
The findings revealed no significant difference ($p>0.05$) in the body weight and testicular weight in the treatment groups compared with the control. The histology showed normal seminiferous tubules in the control group but the testes in the group treated with castor oil showed ulceration of the tunica vaginalis, fibrosis, degeneration of the seminiferous tubules and interstitial congestion. The group treated with castor oil and ginger showed tunica vaginalis without ulceration, normal seminiferous tubules and vascular congestion. The group that received ginger only showed normal seminiferous tubules and interstitial vascular congestion.
The findings demonstrate some potential protective effects of Ginger against Castor oil- induced testicular damage.

Keywords: Aqueous, Extract, Ginger, Castor oil, Testicular Damage, Wistar rats

Introduction
Virtually all plants have medicinal effects, though at extreme doses they can be toxic or lethal to the body (1).
The medicinal effects of plants are being exploited on daily basis all over the world. Before the start of investigations into the chemical constituents of plants in this modern time, there was little or no knowledge of the mechanisms by which these plants exert their effects. Since they affect the systems of the body, some have been reported to affect fertility in males and females (2,3).
Castor oil is a vegetable oil obtained from castor seed. It is a colourless to very pale yellow liquid with a mild or no odour or taste. It has a high boiling point of about 313^{o}C. It is a triglyceride with many fatty acid chains, of which ricinoleic acid is the most abundant and active component responsible for its anti-inflammatory effect (2,4). The other significant fatty acids are oleic acid and linoleic acid (2,4).
Further investigations have revealed that castor oil has anti-fertility effects on female guinea pigs (4), contraceptive effects on women (5) and reversible adverse effects on the reproductive functions of male Wistar rats at a dosage of 40mg/kg (2).
Ginger is a perennial reed-like plant with annual leafy stem, about a meter (3-4 feet) tall. Its thick underground rhizome is consumed whole as a delicacy, medicine or spice. It has been used in traditional medicine to aid digestion, and treat stomach upset, nausea, diarrhea and inflammatory conditions such as arthritis (6). Kamtchouing et al., (7) reported that ginger possesses androgenic activities. Its antioxidant activity was reported by Sekiwa et al., (8). All its major active constituents, zingerone, gingerdiol, gingerol, zingiberene and shogaols possess antioxidant properties (9). Antioxidants protect the DNA and other molecules from oxidation and damage and can improve sperm quality and consequently increase fertility rate in men (10).
The above described plants, ginger (*Zingiber officinale*) and castor oil (*Ricinus communis*) are the plants of study. Based on reports on the effects of both plants on the testes (2,3), this study was done to confirm the role of ginger on castor oil-induced testicular damage.

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Materials and Methods

Collection of Materials and Preparation of Extract

Ginger rhizomes was bought from Uselu market in Benin City, Edo State, Nigeria. Samples were identified in the Department of Pharmacognosy, University of Benin. They were peeled, chopped to pieces, air-dried and grounded. Thereafter, they were subjected to aqueous extraction using the Soxhlet apparatus. The extracted ginger yielded 150g. Castor seeds were bought from a market in Ekuoma village, Delta State. Samples were also identified in the Department of Pharmacognosy. They were peeled, air-dried, grounded and subjected to methanolic extraction using the Soxhlet apparatus and 382g of the oil was obtained as the yield.

Experimental Animals

Twenty (20) adult male Wistar rats were used for this study. The rats were obtained from the animal house in the Department of Animal and Environmental Biology (AEB), University of Benin. They were then conveyed to the animal house in the Department of Anatomy, where they were kept in cages at room temperature. The animals were allowed to acclimatize for 2 weeks, during which they were given livestock grower's mash and water ad libitum. After acclimatization, the rats were divided into four (4) groups, one control group, A, and three experimental groups, B, C and D. Each group had four rats. The control group was given equivalent volume of 2.5% tween 80. Group B was given 200 mg/kg body weight of castor oil, using 2.5% tween 80 as an emulsifier. Group C was given 200 mg/kg body weight of castor oil and 250 mg/kg body weight of ginger. Group D received 250 mg/kg body weight of ginger only. The extracts where administered orally, and the duration of the experiment was ten weeks. The body weights of the animals were noted before and after the administration of extracts.

Histology

Fixation: After ten weeks of administration, the animals were anesthetized with chloroform and then sacrificed. The testes of the animals in the control and experimental groups were extracted and weighed. They were then fixed in 10 % formal saline for 24 hrs.

Embedding: After fixation, the testes were passed through grades of alcohol, 50 %, 70 %, 90 % and 100 % for dehydration. This lasted for 4 hrs, 1 hr in each grade. The tissues were cleared in xylene for 1 hr. This was to remove the alcohol present. They were then infiltrated with molten paraffin wax in an oven at 50-65^{0}C, and then embedded in an embedding mould. Thereafter, they were removed and allowed to cool. The embedded tissue blocks were trimmed and mounted on wooden blocks for sectioning. The sectioned tissues using a rotary microtone were floated in a water bath and picked with glass slides for staining.

Staining procedure: Stainig was done using Haematoxylin and eosin following these steps:

Clearing (removal of wax) in xylene for 3-5 mins and hydration by passing slides through descending grades of alcohol (100 %, 90 %, 70 % and 50 %) for 3-5 mins. The slides were then rinsed in a running tap water for 30 secs and rinsed in distilled water. They were stained with haematoxylin for 5-15 mins and then differentiated in 1% acid alcohol for 30 sces. They were rinsed in a running tap water for another 30secs. After this, they were rinsed in a bluing agent for 1 min, rinsed in a running tap water again for 1 min and counter stained with eosin for 30 mins. The stained slides were later rinsed in 90 % and 100 % alcohol for 5 mins each, and cleared with xylene. The slides were mounted with Canada balsam and covered with glass slips.

Statistical Analysis

Data were analyzed using ANOVA (F-ratio) and ($p<0.05$) was accepted as significant, while ($p>0.05$) was accepted as insignificant.

Results

P*hysical findings*

The results of the initial and final mean body weight of the animals are as presented (Table 1) and the mean testicular weight values of the animals (Table 11):

Table I: Showing Initial and Final Mean Body Weight Values and Standard Errors of Mean of the Experimental Animals

Groups	Initial weight (g)	Final weight (g)	F- ratio
A	275 ± 10.41	287.5±11.99 [a]	0.1953
B	248 ± 18.88	260±17.03 [a]	
C	255±27.23	265±25.38 [a]	
D	270±32.40	280±20.99 [a]	

Table II: Showing The Mean Testicular Weight Values and the Standard Errors of Mean of all the groups.

Group	Mean ± SEM	F ratio ($p<0.05$)
A	1.69 ± 0.08^a	0.1671
B	1.65 ± 0.29^a	
C	1.68 ± 0.21^a	
D	1.70 ± 0.22^a	

Histological findings

The testes of the animals in the control group showed a normal tunica vaginalis enclosing the seminiferous tubules (fig 2). The seminiferous tubule was enlarged to show the layers of spermatocytes in their normal sequential maturation (fig 3). The testes of the animals in group B (castor oil-treated only) showed ulceration of the tunica vaginalis, fibrosis and degeneration of the seminiferous tubules (fig 4). The enlarged seminiferous tubule showed an interstitial congestion (fig 5). The testes of the animals in group C (castor oil and ginger-treated) showed tunica vaginalis without ulceration similar to the normal, vascular congestion and predominantly normal seminiferous tubules without degeneration (fig 6). The enlarged seminiferous tubules showed sequential maturation of spermatocytes (fig 7). The testes of the animals in group D (ginger-treated only) showed normal tunica vaginalis enclosing the seminiferous tubules and interstitial vascular congestion (fig 8). The seminiferous tubule was enlarged to show layers of spermatocytes and their order of maturation (fig 9).

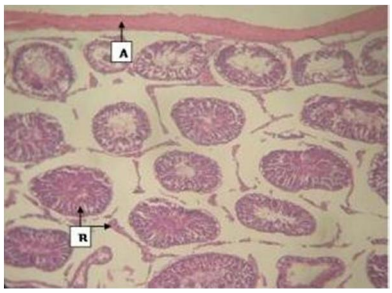

Fig. 2: Normal testis showing tunica vaginalis (A) enclosing seminiferous tubules (B) [H&E x100]

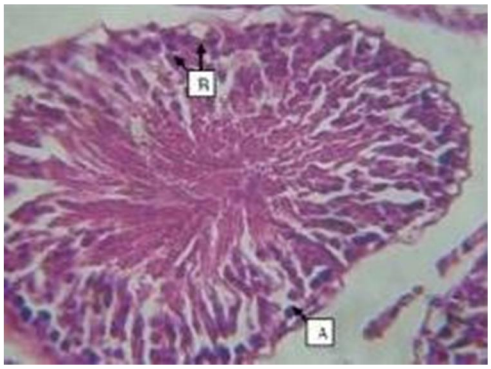

Fig. 3: Normal testis showing seminiferous tubule (A) containing layers of spermatocytes (B) in normal sequential maturation [H&E x1000]

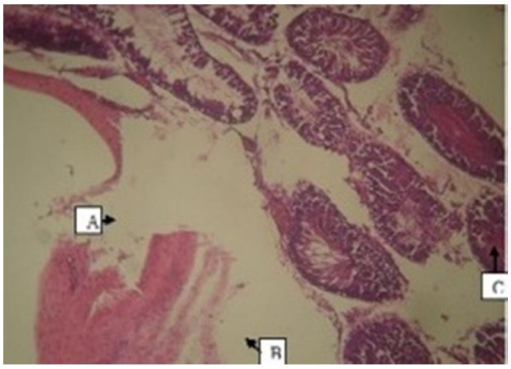

Fig. 4: Castor oil treated testis showing focal tunica ulceration (A) and fibrosis (B) and focal tubular degeneration (C). [H&E x100]

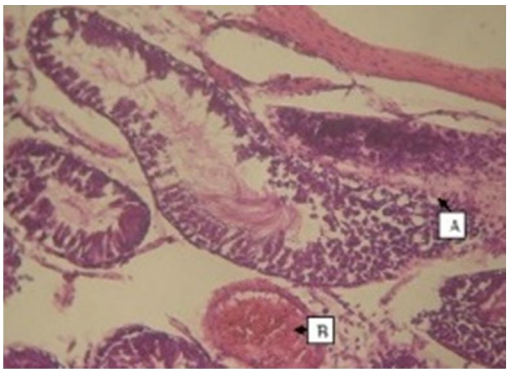

Fig. 5: Castor oil treated testis showing focal tubular degeneration (A) and interstitial congestion (B) [H&E x400]

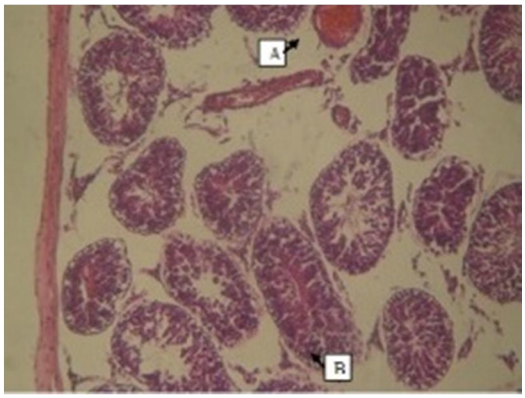

Fig. 6: Castor and ginger treated testis showing vascular congestion (A) and predominantly normal seminiferous tubules (B) [H&E x100]

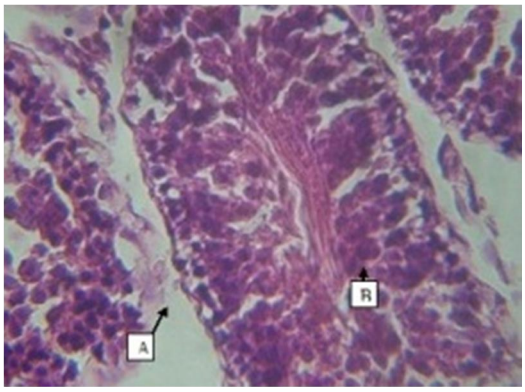

Fig. 7: Castor and ginger treated testis showing normal seminiferous tubule (A) with layers of spermatocytes (B) [H&E x1000]

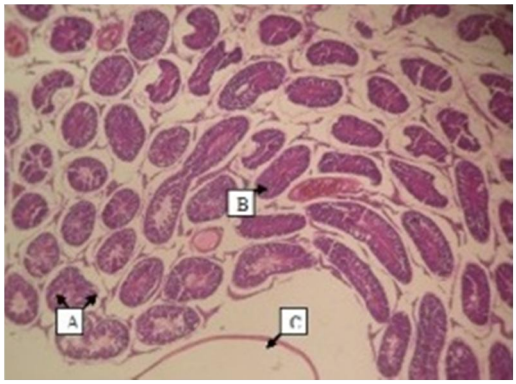

Fig. 8: Ginger treated testis showing normal seminiferous tubules (A), interstitial vascular congestion (B) and a surrounding tunica vaginalis (C) [H&E x40]

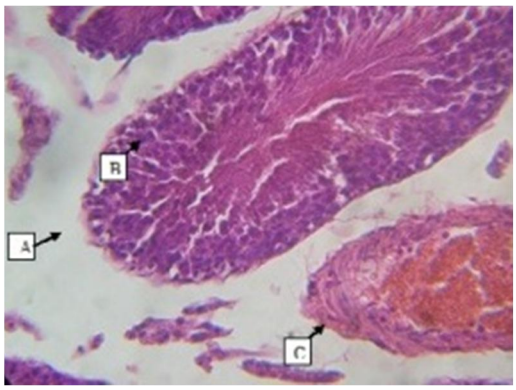

Fig. 9: Ginger treated testis showing normal seminiferous tubules (A) containing layers of spermatocytes (B), and interstitial vascular congestion (C) [H&E x400]

Discussion

Castor oil from castor seed (*Ricinus communis*) contains predominantly ricinoleic acid. The other components are oleic and linoleic acids. They are all fatty acids. The ricinoleic acids has a hydroxyl group (OH^-) as its functional group, which makes it unusually polar and consequently generates free oxygen radicals amongst other molecules such as superoxide ion (O^{-2}) and nitrogen oxide (NO) that also produce free oxygen radicals (11). These free oxygen radicals also known as reactive oxygen species (ROS) are highly reactive and are produced naturally in organisms, but they are restricted within cellular compartments and counterbalanced by the presence of natural antioxidants, such as glutathione, glutathione peroxidase, superoxide dismutase, vitamin E and C. They do this by scavenging the free oxygen radicals (11,12). Cellular damage occurs when there is an imbalance between the generation of free oxygen radicals and the scavenging activities. In the testis, the excessive generation of these reactive oxygen species

up to a level that exceeds the critical level can overpower the natural antioxidant defense plan of the spermatozoa and cause oxidative stress (13,14). Castor oil is one substance that can raise the natural level of the reactive oxygen species by virtue of the presence of ricinoleic acid. In clear contrast to castor oil, ginger (*Zingiber officinale*), which prevents tumor, inflammation, apoptosis (programmed cell death), hyperglycemia and lipidemia (15) is a strong antioxidant that inhibits the production of free oxygen radicals and is also considered as safe herbal medicine that possesses few or no adverse effects (15). Owing to its antioxidants property, ginger extracts have been found to significantly reduce lipid peroxidation by maintaining the activities of antioxidants enzymes like glutathione peroxides, superoxide dismutase and catalase in rats (16), thereby protecting the cells from degeneration.

In the present study, the administration of castor oil to group B, castor oil and ginger to group C and ginger only to group D produced results that are supported by some reported findings. There was no significant change ($p>0.05$) between initial and final body mean weight of all the groups compared with control. There was also no significant difference ($p>0.05$) in testicular weight when the experimental groups were compared to the control. This is supported by Arash et al. (3), who reported that there was no significant difference in the testicular weights of rats that received ginger when compared to control. The histopathological result of the rats in group B revealed ulceration of the tunica vaginalis, fibrosis and degeneration of the seminiferous tubule. This is supported by Raji et al. (2), who reported that there was disorganization of the cytoarchitecture, disruption of the seminiferous tubule and erosion of the germinal epithelium in the testes of rats that received castor oil (*Ricinus communis*). The histopathological result of the rats in group C revealed no ulceration of the tunica vaginalis, vascular congestion and the presence of predominantly normal seminiferous tubule without degeneration. This is supported by Amr and Hamza et al. (17), who reported that ginger had protective effects against cisplatin-induced testicular damage and oxidative stress in rats by increasing the activities of the testicular antioxidant enzymes.

The histopathological result of the rats in group D showed normal tunica vaginalis, seminiferous tubules and interstitial vascular congestion. This report is supported by Kamtchouing et al. (7), Arash et al. (3) and Morakinyo et al. (18), who reported that ginger possesses antioxidant and androgenic properties, and hence beneficial to the testes. The properties of ginger are due to the presence of gingerol, gigerdiol, zingerone, zingiberene and shogaols (9,19).

In conclusion, this study has shown that ginger has protective effects on castor oil-induced testicular damage based on the results obtained.

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