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RESEARCH ARTICLE

Protective Effect of Tahitian Noni Juice on the Reproductive Functions of Male Wistar Rats Traeted with Cyclophosphamide

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ABSTRACT

The effects of Tahitian noni juice (TNJ), vitamin C and vitamin E on male reproductive functions in cyclophosphamide-treated wistar rats were compared. Thirty five male wistar rats with mean body weight of 180 ± 24.3 g were randomly divided into five groups. Group one were treated with cyclophosphamide (50 mg/kg, i.p.) while, group two were treated with TNJ (10 ml/kg, i.p.) and cyclophosphamide. Group three were treated with TNJ (10 ml/kg, i.p.) alone. Group four and five were treated with cyclophosphamide and either of vitamin C (100 mg/kg) or E (1 mg/kg) respectively. All drugs were administered for four weeks. In this study, mean weight gain, sperm motility (SM), live-dead ratio (LDR), epididymal sperm counts (ESC), percentage sperm abnormality, as well as, testicular histological changes were determined. Data were expressed as mean ± standard deviation. The ESC, SM and LDR were compared with analysis of variance (ANOVA). Median score for the histologic changes was analyzed using Wilcoxon sign rank test. The mean weight gain was higher in Groups 2 and 3 compared with other groups (p<0.05). Similarly, the SM was higher in rats treated with TNJ compared with other groups (p<0.001). The LDR, ESC, and testicular histologic scores did not differ between the five groups of rats. However, the percentage sperm abnormality was lower in groups 2 and 3 (p<0.05). In conclusion, TNJ improved weight gain and protected against adverse effect of cyclophosphamide on sperm motility and abnormalities.

Keywords: *Tahitian Noni, Cyclophosphamide, Testis, Rats, Cytoprotective*

Antitumor drugs have achieved lasting clinical remission in many patients with lymphoreticular tumors and other malignancies. With this therapeutic success comes the great concern about persistent or delay toxicities of cancer chemotherapy in long-term survivors, especially the children [1]. For instance, antitumor drugs have been shown to have profound and lasting effect on the testis and the ovary [2]. Germ cell production and endocrine function may both be altered with the magnitude of the effect related to the age, pubertal or menstrual status of the patients, as well as the particular drug, dosage or combination administered [3]. In a long-term study of young adult male survivors of acute lymphoblastic leukemia, male fertility was reportedly poor for at least ten years [2]. The primary testicular lesions caused by antitumor drugs include depletion of the germinal epithelia lining of the seminiferous tubules with increased number of germ cell apoptosis [1]. Other changes caused by antitumor drugs include decreased sperm counts with increased percentage of tail and head abnormalities [4]. In addition, increases in both pre- and post-implantation embryonal losses have been observed following treatment with antitumor drugs [5].

There are still contrasting views on the reversibility of the effects of antitumor drugs on male fertility. While most studies showed that the effect is transient and only limited to the immediate post treatment period [6], other studies have reported a more persistent effect on spermatogenesis [7]. Long term and late effects may become evident with puberty and may persist for month or years after treatment ends. Genotoxicity may be one reason accounting for the long term or late effects. This occurs primarily due to alteration of the phosphate backbone sugar or base modification such as alkylation, cross links or formation of bulky DNA-adducts [8, 9].

Table 1. Primary and secondary sperm abnormalities, testicular histology score and mean body weight following intraperitoneal injection of cyclophosphamide

Semen and testicular parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
Primary sperm abnormalities (%)	5.4 ± 2.3[a]	2.3 ± 1.2[b]	2.2 ± 1.4[b]	5.2 ± 3.2[a]	4.8 ± 2.3[a]
Secondary sperm abnormalities (%)	7.6 ± 1.3[a]	3.6 ± 1.8[b]	3.1 ± 2.3[b]	6.6 ± 2.1[a]	6.3 ± 2.5[a]
Median Testicular Histological scores	1	1	1	1	1
Mean body weight at start of experiment (G)	182.9 ± 7.8[a]	180.0 ± 5.7[a]	178.6 ± 8.4[a]	180.0 ± 7.5[a]	181.4 ± 6.6[a]
Mean body weight at the end of experiment (G)	201.6 ± 29.8[a]	216.3 ± 36.4[a]	229.6 ± 35.3[a]	196.7 ± 8.5[a]	208.4 ± 43.8[a]
Mean body weight gain (G)	18.7 ± 6.5[a]	36.3 ± 5.9[b]	51.0 ± 4.8[c]	16.7 ± 6.6[a]	23.0 ± 7.5[a]

Following intraperitoneal injection of cyclophosphamide cyclophosphamide (50 mg/kg), the rats were treated with either distil water (Group 1), Tahitian Noni (Group 2), Vitamin C (Group 4) and Vitamin E (Group 5). Group 3 rats were only treated with Tahitian Noni Juice.
Values with different superscript are statistically significant ($p< 0.05$)

Several studies have suggested that dietary supplementation with antioxidant can influence the response to chemotherapy as well as the development of adverse effects that result from antitumor drugs [10, 11]. This is thought to be associated with the detoxification of reactive oxygen radical and the inhibition of the activity of topoisomerase [12]. For instance, selenium has been reported to have antitumor activity against several tumors as well as protecting against the nephrotoxic effect of cisplatinum [13]. Similarly, supplementation with vitamin C has been suggested as a potential protective agent against the side effects of antitumor drugs [14, 15]. However, there is still continued effort in search of an agent that provides the best protection against antitumor long term adverse effects.

Morinda citrifolia (Noni) have been reported to have a broad range of health benefits for cancer, infection, arthritis, diabetes, asthma, hypertension and pain [16]. A number of major components have been identified in Noni plant including scopoletin, potassium, vitamin C, anthraquinones, carotene, vitamin A, caproic acid and a putative proxeronine [17]. Noni was reported to enhance the therapeutic efficacy of anticancer drugs such as prednisolone and taxol [16]. There is increasing anecdotal report on the benefits of Noni in cancer patients. Daily intake of Noni was reported to improve the quality of life of patients undergoing chemotherapy or radiotherapy [18]. This study was therefore, designed to evaluate if concurrent administration of Noni in rats treated with cyclophosphamide will protect against the cytotoxic effect of the antitumor drug in the male wistar rats. In addition, we compared the cytoprotective effect of Noni to that of vitamin C and vitamin E.

MATERIALS AND METHODS

Thirty–five sexually-matured male Wistar rats with mean body weight of 180 ± 24.3 g were used. They were housed in a group of seven in metal cages bedded with wood shavings and maintained according to the National Institute for Health (NIH), USA, Guidelines for Laboratory Animals. In addition, they were fed with pelleted grower ration (Guinea feeds Ltd, Benin, Nigeria) ad-libitum, while water was also provided ad-libitum. The protocol for this study was approved by the Ethical Committee of the College of Veterinary Medicine, University of Agriculture, Abeokuta.

This study involved a randomized blinded design, the observer not aware of the treatment administered. The rats were randomly divided into five treatment groups. Rats in group one were treated with 50 mg/kg intra-peritoneal injection of 2% cyclophosphamide (Cycram®, Korea United Pharmacy, Chungnam, Korea) weekly for four weeks and 2 ml of distilled water orally. Rats in group two were treated with 10 ml/kg Tahitian Noni (TNI) (Tahitian Noni Int., Germany) and intraperitoneal injections of 2% cyclophosphamide. Rats in group three were treated with 10 ml/kg Tahitian Noni juice. Rats in groups four and five were treated with 100 mg/kg vitamin C (Ascorbitone®, Archy Pharmaceuticals, Lagos, Nigeria) and 1 mg/kg vitamin E (Evitol®, Teva Pharmaceuticals) respectively, as well as 2% cyclophosphamide. Cyclophosphamide was administered once weekly, while all treatments lasted for four weeks. All oral drugs were administered into the drinking water.

The rats were weighed weekly, while food intake was determined daily till the end of the experiment. A period of one week was allowed after the administration of the last injection of the cyclophosphamide before the rats were euthanized. Rats were euthanized with Halothane. Following euthanasia, a ventral midline skin incision was made to access the abdomen; the testis and epididymis were located and removed for the determination of epididymal sperm counts, progressive sperm motility, live-dead ratio and sperm morphology. Thereafter, the testes were fixed in Bouin's fluid for histopathology.

Epididymal sperm count was determined using the Neubauer haemocytometer chamber after dissecting the caudal epididymis and squeezing its content into warm sterile distilled water. The motility of the spermatozoa was assessed as the percentage of the rapidly-

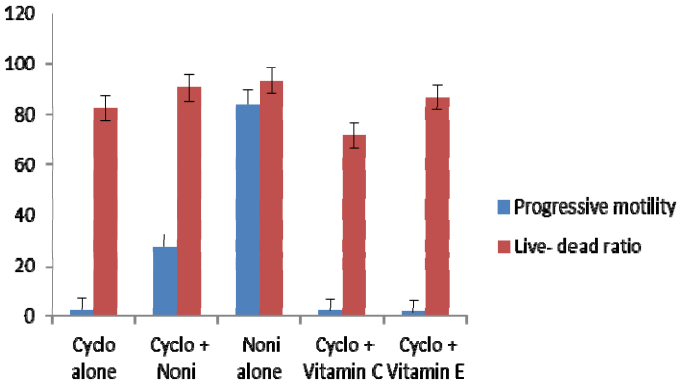

Fig 1. Progressive motility and percentage live- dead ratio in rats treated weekly with intraperitoneal injections of cyclophosphamide (50 mg/kg) and daily administration of distil water, Tahitian Noni (10 ml/kg), Vitamin C (100 mg/kg) and Vitamin E (1 mg/kg). The control group comprises of rats that were not treated with cyclophosphamide but are daily treated with Tahitian Noni (10 ml/kg) orally.

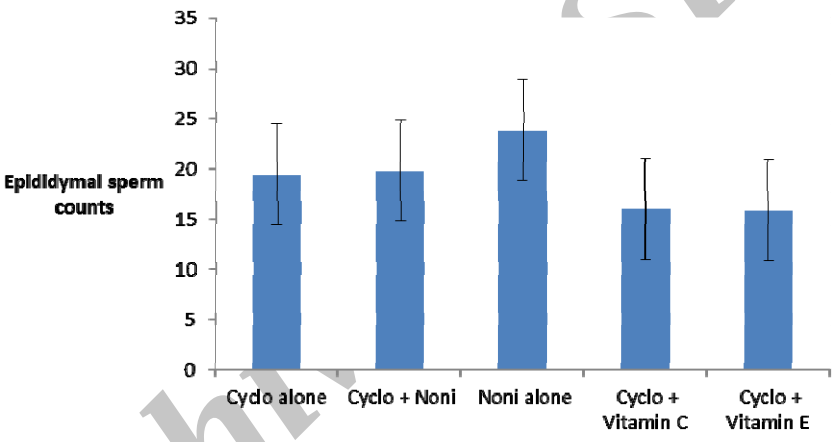

Fig 2. Epididymal sperm concentration in rats treated weekly with intraperitoneal injections of cyclophosphamide (50mg/kg) and daily administration of distil water, Tahitian Noni (10 ml/kg), Vitamin C (100 mg/kg) and Vitamin E (1 mg/kg). The control group comprises of rats that were not treated with cyclophosphamide but are daily treated with Tahitian Noni (10 ml/kg) orally.

progressive motile sperm by two independent blind assessors (TAA and OTA), while the live–dead ratio was determined using the eosin-negrosin staining method [19]. Thereafter, sperm abnormalities were classified as primary or secondary. The histology of the testes was evaluated and scores ranging from 0-4 was assigned to observable changes of the testes by two blinded assessors [20].

Data were expressed as mean ± standard deviation. Epididymal sperm concentration, progressive sperm motility and live-dead ratio were compared with analysis of variance (ANOVA). Median score for the histologic evaluation and sperm abnormalities were analyzed using Wilcoxon sign rank test.

RESULTS

The mean weight gain was significantly (p<0.05) higher in the rats in groups 2 and 3 compared with the other groups. Also, the mean weight gain was significantly higher in rats in group 3 compared with rats in group 2 (Table 1). The result of the progressive sperm motility and the live-dead ratio is shown in Fig 1. The progressive sperm motility was significantly higher (p<0.001) in rats that were treated with TNJ alone compared with other groups (Fig 1). Similarly, sperm motility was significantly (p<0.05) higher in the rats that were treated daily with TNJ and cyclophosphamide compared to those that were treated with distilled water, vitamin C or E. In addition, there was no significant difference in the sperm motility between rats treated with either vitamin C or E along with intraperitoneal injection of cyclophosphamide (Fig 1). The live-dead ratio was significantly (p< 0.05) lower in rats that were treated daily with oral vitamin C compared with the other treatment groups. However, the epididymal sperm concentration (ESC) did not differ between the five groups of rats (Fig 2). The ESC tended to be highest in rats that were treated with TNJ and those treated with

TNJ and cyclophosphamide, and lowest in rats treated with either vitamin C or E. In addition, the histologic scores of the rats did not differ significantly among the five treatment groups; while, the percentage sperm abnormality was significantly (p<0.05) lower in the rats in groups 2 and 3.

DISCUSSION

The result of this study showed that concurrent administration of TNJ in rats receiving intraperitoneal injection of cyclophosphamide significantly improved the weight gain as well as the measured sperm indices. In addition, the protection provided by oral administration of TNJ was found to be significantly better than that of vitamin E or C. The use of cyclophosphamide for the treatment of cancer in male patients was reported to increase the incidence of oligospermia and azoospermia, with resultant male infertility [3]. In addition, there is moderate to severe reduction in body weight with alteration of epididymal functions. In this study, administration of 50 mg/kg of cyclophosphamide was characterized by decreased weight gain and sperm motility without significant histologic changes in the testis. The cytotoxic effects of cyclophosphamide in male germ cells are mediated through the induction of DNA adducts, DNA strand breaks, and cross-links [9]. The genotoxic effect of cyclophosphamide on male germ cells was reported to be dependent on the stage of the germ cell development during which the cells are exposed to the drug [21]. Post-meiotic germ cells are more susceptible to the effects of cyclophosphamide. The response of the male germ cells to the genotoxic damage relies in part on the presence and functionality of stress response mechanisms during germ cell development [22]. This has been shown to be dose-dependent and time-specific. Differences in the response of different stages of male germ cells to cyclophosphamide exposure might have accounted for the reasons why no significant changes were observed in the histology of the testis of the rats in this study.

Cytostatic metabolites and reactive oxygen species are produced during cyclophosphamide metabolism. This reaction is catalyzed by cytochrome P450, peroxidases and lipooxygenases [23]. Oxidative stress leads to injury of organs such as the bone marrow, kidney, urinary bladder and reproductive system. Several compounds have been investigated to prevent the production of free radicals and alleviate lipid peroxidation in different tissue following administration of cytotoxic chemotherapeutic agents. Vitamin E was reported to possess protective effect against lipid peroxidation induced by adriamycin in rats [24]. The effect was found to be dependent on the timing and dosage of the vitamin E administered. Similarly, vitamin C was reported to partly protect the DNA damage induced by cisplatinum at high concentration [14]. In this study, both vitamin C and E appeared not to significantly protect the rats following cyclophosphamide injection, while TNJ significantly protected the rats against the adverse effect of cyclophosphamide based on the outcome of the sperm motility and percentage of abnormal spermatozoa. This suggests that TNJ possess a better cytoprotective effect than either of vitamin C or E. In a previous in-vitro study of the antioxidant activity of Tahitian noni juice, TNJ was found to have better antioxidant effect than grape seed extract or vitamin C [17]. The better cyto-protective effect exhibited by Tahitian noni juice may be due to its stronger antioxidant effect.

In conclusion, the result of this study showed that Tahitian noni juice improved the quality of life of rats treated with cyclophosphamide by improving the weight gain and protecting against the adverse effect of the drug on sperm motility, live-dead ratio, as well as reducing the number of both primary and secondary sperm abnormalities. This finding thus suggests that Tahitian noni juice may improve fertility in cancer patients receiving antitumor drugs. However, studies on animals with known tumor, to evaluate if the administration of noni juice will not interfere with the efficacy of the antitumor drug are required.

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