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Modulatory Roles of Exogenous Antioxidants on Nevirapine Induced Effects on Sperm Quality

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Rotimi Kunle*[1], Obamiro Kehinde[2] and Ikumawoyi Victor[1]

1 Department of Pharmacology, Therapeutics and Toxicology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.

2 Department of Pharmacy, School of Medicine , University of Tasmania

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ABSTRACT

Objective: This study evaluated the protective anti-oxidative effect of antioxidants on nevirapine induced effects on sperm parameters in rats. **Methods:** Male albino Rats with an average weight of 140 g were divided into 6 groups with 8 animals per group. A group received distilled water 10 ml/kg for two weeks followed by Nevirapine (6 mg/kg) for 60 days while another group received distilled water 10 ml/kg for two weeks and continued for 60 days with the same dose of distilled water. Other groups received doses of antioxidants Sorghum bicolor (10.7 mg/kg), Vitamin C (8 mg/kg), Vitamin E (5 mg/kg), Vitamin E (5 mg/kg) + C (8 mg/kg) respectively for 2 weeks. After two weeks a combination of these antioxidants and Nevirapine (6 mg/kg) was then administered to the respective groups for 60 days. All treatments were p.o, Sperm samples were collected from the epididymis to achieve count and motility and morphological analysis. **Results**: The results obtained showed a significant ($p<0.05$) higher levels of total sperm abnormalities for the group treated with Nevirapine and Vitamin E compared with the control group, Nevirapine and Sorghum bicolor capsule group. Results for the Vitamin E and Vitamin C combinations show a statistical significant ($p<0.05$) lower level of total sperm abnormalities compared with Vitamin C and Vitamin E group. **Conclusion:** Observations from this study suggest potential positive modulatory effects of a combination of Vitamin E and Vitamin C in limiting nevirapine induced total sperm abnormalities. Sorghum bicolor capsule and vitamin E and C alone however show less potential positive effects.

INTRODUCTION

The use of antiretroviral is a core component of HIV/AIDS chemotherapy which entails a continuous use of standard combinations of non nucleoside reverse transcriptase inhibitor, nucleoside reverse transcriptase inhibitor, protease inhibitors, fusion inhibitors and other classes of antiretrovirals. Due to the long duration of the therapy, toxicity is usually inevitable. One of the most common antiretroviral that has been associated with toxic reactions is nevirapine[12]. Nevirapine is a non nucleoside reverse transcriptase inhibitor that is usually used in combination with nucleoside reverse transcriptase inhibitor as a component of a highly active antiretroviral regimen.

A study by Adaramoye et al shows a significant difference in sperm parameters in rat exposed to 18 and 36 mg/kg body weight of nevirapine, compared with the control. This finding imply that chronic dose of nevirapine may lead to sperm abnormalities. One of the postulated mechanism of nevirapine induced toxicities is via the induction of oxidative stress seen as an increase in the oxidative stress parameters in rats and also in individual on nevirapine based regimens [8,13,18,19].

Sorghum bicolor is a plant grown across America, Africa and Asia in areas that are too hot and dry to grow other grains [16]. It is known to have a high content of antioxidants including simple phenolic acids, as well as polyphenols particularly 3- deoxyanthocyanidins such as luteolinidins and apigenidins [10]. The highest content of these antioxidant compounds are present in sorghum based beer and contribute to the inhibition of lipid peroxidation during meshing and boiling [16]. The ethanol extract of *Sorghum bicolor* leaf sheath was shown to have a high chemical antioxidant capacity of 37,622 µM Trolox equivalent per gram; the strongest capacities for quenching free radicals were seen for hydroxyl-free radicals and super oxide anions[16]. Also an ethanolic extract of *Sorghum bicolor* leaf sheath shows a potential protective role against cadmium- induced oxidative stress in rats [23]. This suggest *Sorghum bicolor* capsule, may offer protective effects against Nevirapine induced oxidative stress.

Kolaviron and vitamin C has been shown to prevent testicular toxicity induced by chlorambucil [11], vitamin E and C has also shown to positively modulate the effects of rifampicin on sperm quality [24]. Recent studies have also shown that antiretroviral drugs induce oxidative stress via generation of oxidative radicals which may be associated with their toxicological effects [29].

Since seminal plasma antioxidant status plays a role in abnormal sperm quality [4] and given that the antioxidant system prevent tissue damage caused by oxidative radicals during a period of persistent oxidative stress, it may be important to evaluate the role of this system in ameliorating drug induced sperm abnormalities.

The aim of this work was to evaluate the protective anti-oxidative effects of different exogenous antioxidants (*Sorghum bicolor* capsule, vitamin C and vitamin E) in alleviating nevirapine induced effects on sperm morphology, motility and abnormality. This investigation into the possible tissue protective roles of these antioxidants will be done by measurement of sperm parameters

MATERIALS AND METHODOLOGY

3.1 Chemicals

NVP (Viramune) produced by Boehringer Ingelheim (Germany) was used for this study and obtained from PEPFAR (President's Emergency Plan for AIDS Relief) Clinic, Lagos University Teaching Hospital, Vitamin C, Vitamin E and *Sorghum bicolor* capsule were obtained from Health plus Pharmacy which is an accredited pharmacy in Lagos, Nigeria.

3.2 Materials

Oral cannula, Glass wares, Pipette devices, Test tube racks, Syringe and Needle (Agary Pharmaceuticals Ltd. China), plain sample bottles, complete set of dissecting instruments

3.3 Animals used

The animals used in this study were 4-6 weeks old male albino rats with an average weight of 100 g. They were obtained from Laboratory Animal Centre of the College of Medicine, University of Lagos, Idi-Araba. They were housed and kept in standard environmental conditions having access to standard rodent feed and clean water ($_{P}$H 7.0) ad libitum and acclimatized for a period of two weeks before experimental procedures.

3.4. Study Design

The animals were divided into six groups, with each containing eight rats each. The antioxidant group were pretreated with *Sorghum bicolor* capsule (10.7 mg/kg),Vitamin C(8 mg/kg) and

Vitamin E (5 mg/kg) for two weeks before the administration of Nevirapine. The first group of rat was designated control and received normal saline (10 ml/kg/day p.o) throughout the treatment period. The second group was received Nevirapine (6 mg/kg/day p.o) for a period of 60 days. The third group was given jobelyn (10.7 mg/kg/day p.o) for two weeks then a combination of Nevirapine (6 mg/kg/day p.o) and *Sorghum bicolor* (10.7 mg/kg/day p.o) for 60 days. The fourth group was given Vitamin C (8 mg/kg/day p.o) for two weeks then a combination of Nevirapine (6 mg/kg/day p.o) and Vitamin C (8 mg/kg/day p.o) for 60 days. The fifth group was given Vitamin E (5 mg/kg/day p.o) for two weeks then a combination of Nevirapine (Nevirapine 6 mg/kg/day p.o) and Vitamin E (5 mg/kg/day p.o) for 60 days. The last group was given a combination of Vitamin C (8 mg/kg/day p.o) and Vitamin E (5 mg/kg/day p.o) for two weeks followed by a combination of Nevirapine (6 mg/kg/day p.o), Vitamin C (8 mg/kg/day p.o) and Vitamin E (5 mg/kg/day p.o) for 60 days. The dose of Nevirapine was based on the human therapeutic dose of Nevirapine[20].

Sperm analysis

Seminal fluid obtained from male animals across the different treatment groups was analyzed to determine sperm motility, count, and morphology using the methods of Cheesbroughand Ogli et al Seminal fluid was collected according to the method of Ogli et al.

Statistical analysis

All values were expressed as the mean±SD of seven animals per group. Data were analyzed using one-way analysis of variance (ANOVA) followed by the Turkey comparison test using Graphpad prism. Values were considered statistically significant at $p < 0.05$.

RESULTS

Effect of *Sorghum bicolor* extract and Other Exogenous Antioxidant on sperm Parameters of Nevirapine Treated Rats

There was a statistical significant ($p<0.05$) higher levels of total sperm abnormalities for the group treated with nevirapine and vitamin E compared with the control group, nevirapine and *Sorghum bicolor* group. This may suggest that vitamin E offers no advantage in preventing nevirapine induced sperm abnormalities. Results for the group with vitamin E and vitamin C

combination shows a statistical significant ($p<0.05$) lower level of total sperm abnormalities compared with vitamin C and vitamin E group. However result for sperm count and motility shows no statistical significant ($p<0.05$) variations among the groups.

TEST	Morphology (Mean±SD)	Sperm count (Mean±SD)	Sperm motility (Mean±SD)
CONTROL	0.032±0.008367	5.695×10^7 ±6081221	0.69±0.6519
NEV	0.042±0.01304	5.1498 $\times 10^7$±2852853	0.62±0.1255
Sorghum Bicolor +NEV	0.038 ± 0.01483	5.3×10^7±3137475	0.62±0.02739
VITC+NEV	0.052 ±0.008367	5.675 $\times 10^7$±4383919	0.566±0.06066
VITE+NEV	0.07± 0.01[a,b,c]	5.149 $\times 10^7$±3478577	0.564±0.05899
VITE+VITC+NEV	0.01667 ±0.01155[d,e]	5.583 $\times 10^7$±4389856	0.55±0.08660

NEV = Nevirapine; VITC= Vitamin C; VITE = Vitamin E

Data are expressed as mean ± SD (n =8).

[a] represent results where $p<0.05$ as compared with control

[b] represent result where $p<0.05$ as compared with Nevirapine

[c] represent result where $p<0.05$ compared with *Sorghum bicolor* + Nevirapine

[d] represent results where $p<0.05$ as compared with Vitamin C + Nevirapine

[e] represent results where $p<0.05$ as compared with Vitamin E + Nevirapine

[f] represent results where $p<0.05$ as compared with Vitamin C + Vitamin E + Nevirapine

DISCUSSION

This study was designed to evaluate possible protective/modulatory roles of *Sorghum bicolor* and other nutritional antioxidants in Nevirapine induced sperm toxicity, this is because antioxidant has been shown to be a vital defense against free radical induced infertility[15], which is usually seen as low sperm quantity, reduced motility and abnormal morphology. Generally a

higher antioxidant status is believed to prevent lipid peroxidation in spermatozoa and therefore results in better sperm motility, normal sperm morphology and higher sperm count.

The result of our study is consistent with that of Adaramoye et al which shows that nevirapine lead to no detrimental effect on total sperm quantity[1], indicating a lack of detrimental nevirapine effects on sperm quantity, the data also shows no statistical significant higher sperm quantity in the antioxidant groups. However nevirapine caused significant reduction in sperm motility and viability with a discernable increase in sperm abnormalities in the rats[1].

Evaluation of sperm motility, sperm count and abnormal sperm morphology showed a statistically significant ($p<0.05$) higher total sperm abnormalities for the vitamin E group compared with the control group, nevirapine group and *Sorghum bicolor* group. Vitamin E have been suggested to possess a potential protective ability to prevent lipid peroxidation and also contributing in scavenging reactive oxygen species, An *in vitro* study suggest vitamin E may protect spermatozoa from abnormal morphology and loss of motility[9]. However this study does not substantiate these facts and this may imply that vitamin E may actually contribute towards nevirapine induced total sperm abnormalities. This may be related to the ability of vitamin E to act as pro-oxidants thereby further inducing oxidative stress in treated animals. Due to the fact that higher dosage of antioxidants could become pro-oxidants thereby damaging cellular structure [5,14]. Therefore the cumulative effects of the pro-oxidant roles of vitamin E and nevirapine induced oxidative stress probably result in the higher level of total sperm abnormalities observed in the vitamin E group.

Testicular lipid peroxidation process produced by Nevirapine may damage the membranes of spermatozoa and may lead to rapid loss of intracellular ATP resulting to axonemal damage, decreased sperm viability and increased morphological defects [30,23], since lipid peroxidation is a major culprit of motility and morphological defects observed in spermatozoa, the presence of antioxidant should limit, abolish or prevent this defect, because of their ability to prevent the formation of reactive oxygen species and decrease reactive oxygen species already formed[2]. This assertion has been demonstrated. In a clinical trial, antioxidant supplementation show potency in limiting oxidative stress induced male sperm defects [25]. A statistical significant higher level of total sperm abnormalities in the vitamin E group suggests the destruction of the lipid matrix of the spermatozoa membrane even in the presence of exogenous Vitamin E. This shows that at the

administered dose, Vitamin E shows no potential in improving sperm parameters in the presence of Nevirapine.

Studies demonstrated that *in vivo* exposure to antioxidants improves steroidogenesis by increasing the primary effect of on leydig cell endocrine function along with enhanced circulatory testosterone production and stimulation of spermatogenesis [17]. However for this study there was no statistical significant higher level of sperm count in the antioxidant groups compared with the nevirapine group and this may suggest that exposure of antioxidants offers no beneficial effects in spermatogenesis in male rats.

An imbalance between production of reactive oxygen species (ROS) and seminal antioxidant system results in seminal oxidative stress. Also it has been observed that seminal oxidative stress is responsible for sperm dysfunction and sperm DNA damage in male infertility[3, 31]. However a controlled production of these ROS is important for sperm physiology[32]. The production of high level of ROS by immature germ cells and leukocytes leads to sperm dysfunction which is characterized by loss of motility and abnormal morphology[32].

A statistical significant lower level of total sperm abnormalities was also observed for the group exposed to a combination of Vitamin E/Vitamin C and Nevirapine. This suggest a combination of vitamin C and vitamin E offers significant beneficial effects on nevirapine induced sperm abnormalities compared with lone supplementation with vitamin E or vitamin C alone and also natural source of antioxidant . This result may be a result of the ability of vitamin C and vitamin E to acts synergistically, thereby preventing oxidative attack on spermatozoa. *In vitro* and animal studies have suggested that vitamin C enhances the antioxidant potential of vitamin E by reducing tocopheryl radicals [5,6,27]. Vitamin C might reduce the tocopheroxyl radical produced from tocopherol during the scavenging of free radicals *in vivo*, which permits a single molecule of α-tocopherol to inactivate many radicals thereby improving the antioxidant properties of vitamin E.

CONCLUSION

Observations from this study suggest potential positive modulatory effects of a combination of vitamin E and vitamin C in limiting sperm abnormalities and may be indicative of inclusion of

antioxidants in nevirapine based ART. *Sorghum bicolor*, vitamin E and C alone however shows less potential positive modulatory roles when compared with vitamin C and vitamin E combination. Further studies are recommended to elucidate the exact mechanism of antioxidant effects and recommended dose in patient on highly active antiretroviral therapy.

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