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The Role of some Medicinal Plants in Reducing Sperm DNA Damage of Male Mice

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Abstract

The aim of this study was to investigate the role of some medicinal plants in reducing sperm DNA damage of male mice. The plants (ginger, *Coriandrum sativum* ,*Hibiscus sabdariffa* and *Garcinia kola*) were subjected to preliminary phytochemical screening to identify the chemical constituents. Adult mice (n=50) were included in the present study. Thereafter, the mice were randomly divided into control (n=10) and experimental (n=40) groups. The control group just received 8ml distilled water daily. However, the experimental 100gm/Kg/BW of ginger, *Coriandrum sativum* ,*Hibiscus sabdariffa* and *Garcinia kola* for 21 consequence days in water. The present study was showed on the preliminary phytochemical analysis result of the aqueous ginger, *Coriandrum sativum*, *Garcinia kola* and *Hibiscus sabdariffa* found that the presence of varying amount of alkaloids, saponins, tannins, cardiac glycosides, cardenoledes, flavonoids steroidal ring, and phlobatanins in the concentrations. Sugar, phlobatanins and tannins were absent in ginger. While alkaloid, phlobatanins and saponins were absent in *Coriandrum sativum* and steroidal ring in *Hibiscus sabdariffa.*While on DNA damage the study found the ginger effect there was 94% of sperm with normal morphology and 3% immature and DNA Damage 3% when in *Coriandrum sativum* there was 91% of sperm with normal morphology and 7.5% immature and DNA Damage 2.5% and the effect of *Garcinia kola* and *Hibiscus sabdariffa* there were 89% ,79% of sperm with normal morphology , 5.5%,14% immature and DNA damage 5.5%,7% respectively compared with control group 85% of sperm with normal morphology and 7%,8% immature and DNA Damage.

Key words: medicinal plants, sperm DNA Damage, male mice.

Introduction

Recently, several studies have indicated an increase in the rates of sperm chromosomal an euploidy, sperm DNA, and chromatin condensation abnormalities in semen samples of male partners from couples with recurrent spontaneous abortion (RSA) compared to fertile controls (World Health Organization,2013). However, on the other hand, other studies have reported that sperm DNA integrity is not associated with unexplained RSA (Bellver J,*et al*,2010). However, some reports questioned the usefulness of antioxidants in the treatment of male infertility (Martin-Du Pan and Sakkas, 1998; Bolle *et al*., 2002; Agarwal *et al*., 2004), and in-vitro effects of antioxidants on sperm DNA integrity are inconsistent (Hughes *et al*., 1998; Donnelly *et al*., 1999). To our knowledge, no evaluation of the effect of oral antioxidant treatment on ICSI clinical outcomes in cases with elevated sperm DNA damage has yet been reported. Oral antioxidant treatment appears to improve ICSI outcomes in those patiens with sperm DNA damage, in whom this treatment reduces the percentage of damaged spermatozoa (Ermanno G,*et al*,2005). Accordingly, it is expected that antioxidant therapy acts as a protective defense against oxidative stress and improve fertility parameters (Zahedi and Khaki, 2014).Khaki *et al*., (2009) reported that ginger extract possess a protective effect against DNA damage induced by H2O2 and enhanced sperm healthy parameters in rats. *Coriandrum sativum* (Linn.), has a very effective antioxidant profile (Panda S *et al* 2009). *Garcinia kola* is now known to contain a high content of biflavonoid compounds and its remarkable bioactivities have been ascribed principally to the possession of these flavaonoids due to their enormous antioxidant activities (Adaramoye *et al*., 2005; Emerole *et al.*, 2005). The calyces, which are rich in phenolic compounds contain gossypetin, glucoside, hibiscin, hibiscus anthocyanin and hibiscus protocatechuic acid, possess diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure and stimulating intestinal peristalsis (Ali and Salih, 1991; Owulade *et al*., 2004). Tseng *et al*., (1996) found that the mechanism of this protective effect was associated with the scavenging of free radicals Hibiscus protocatechuic acid also inhibits lipopolysaccharide-induced rat hepatic damage. For many years, researchers have been studying how to best quantify the amount of abnormal DNA that is present in human spermatozoa and relate the results to fertility(Perreault SD,*et al* 2003). Several techniques can be used to study DNA defects in human spermatozoa. Toluidine blue (TB) staining has been reported to be a sensitive test for incomplete DNA structure and packaging (Talebi AR,*et al* 2012).

Materials and Methods

Phytochemical analyses

Phytochemical analyses includes determination, of alkaloids, saponins, tannins, cardiac glycosides, cardenoledes, flavonoids steroidal ring, and phlobatanins in the aqueous of ginger, *Coriandrum sativum* ,*Hibiscus sabdariffa* and *Garcinia kola* . All of these were determined based on methods of analyses described by AOAC (1990).

Experimental animals

Adult mice (n=50) were included in the present study. The mice were 8 weeks old and weighing 28±3g each. Male

mice were housed in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12h/12h light/ dark cycle prior to experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care. All mice were fed a standard diet. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Thereafter, the mice were randomly divided into control (n=10) and experimental (n=40) groups. The control group just received 8ml distilled water daily. However, the experimental groups split into 1, 2,3and 4 groups each included ten mice. All experimental groups received 100ml/kg/BW of ginger, *Coriandrum sativum ,Hibiscus sabdariffa* and *Garcinia kola* for 21 consequence days in water

Epididymis sperm collection

Sperms from the cauda epididymis were released by cutting into 2 ml of medium containing 0.5% bovine serum albumin.

Sperm DNA Damage Assay

To assay DNA damage aniline blue stain was used. To perform this staining, sperms were stained with AB-eosin. The slides were prepared by smearing10 µL of each raw semen sample. The slides were air-dried and then fixed in 4% formalin for 5 minutes at room temperature, rinsed in water, and stained in 5% aniline blue in 4% acetic acid (pH 3.5) solution for 5 minutes. After 5 minutes, the slides were rinsed in water and stained in 0.5% eosin (Merck) for 1 minute. This was followed by rinsing and air drying of the slides. Under light microscopic evaluation, 300 spermatozoa were counted in different areas of each slide using ×1,000 magnification. Immature sperm were stained dark blue by the eosin counter stain. The percentage of abnormal sperm chromatin condensation was calculated as the ratio of the number of dark-blue sperm to the total number of sperm analyzed (Wong *et al*., 2008).

Result

Phytochemical analysis

The preliminary phytochemical analysis result of the aqueous ginger, *Coriandrum sativum, Garcinia kola* and *Hibiscus sabdariffa* found that the presence of varying amount of alkaloids, saponins, tannins, cardiac glycosides, cardenoledes, flavonoids steroidal ring, and phlobatanins in the concentrations. Sugar, phlobatanins and tannins were absent in ginger. While alkaloid, phlobatanins and saponins were absent in *Coriandrum sativum* and steroidal ring in *Hibiscus sabdariffa* shown in the table (.1).

The effect on sperm DNA damage of mice treated with ginger, *Coriandrum sativum ,Hibiscus sabdariffa* and *Garcinia kola*

Comparison effects of ginger, *Coriandrum sativum ,Hibiscus sabdariffa* and *Garcinia kola* on DNA damage the study found the ginger effect there was 94% of sperm with normal morphology and 3% immature and DNA Damage 3% when in *Coriandrum sativum* there was 91% of sperm with normal morphology and 7.5% immature and DNA Damage 2.5% and *Garcinia kola* and *Hibiscus sabdariffa* there were 89% ,79% of sperm with normal morphology and 5.5%,14% immature and DNA damage 5.5%,7% respectively compared with control group 85% of sperm with normal morphology and 7%,8% immature and DNA Damage (Table.2).

Discussion

The main pharmacological actions of ginger and compounds isolated there from include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic actions. Ginger is a strong anti-oxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Ali *et al*., 2008). Phytochemicals analysis of ginger in the present study was approved by Ugwoke CEC and Nzekwe, U (2010) who found that The results of the phytochemical screening showed that alkaloids, carbohydrates, glycosides, proteins, saponins, steroids, flavonoids and terpenoids were present, while reducing sugars, tannins, oils and acid compounds were absent. When the result of *Coriandrum sativum* was finding by Kanchan, P. K. et al (2012) who found that presence of tannins, terpenoids, reducing sugars, flavonoids, and glycosides and alkaloids were absent in aqueous extract of *Coriandrum sativum.*The result obtained from *Garcinia kola* was similar result presented by Adesuyi,A.O *et al* (2012) who found that The Phytochemical parameters carried out were Flavonoids, Phenol, Alkaloids, Saponin and Tannin. Obouayeba A, P (2014) finding the present of alkaloids, flavonoids, saponins, steroids, sterols and tannins are present in petals of the *H. sabdariffa.* Comparison effects of four plants on DNA damage the study found the ginger effect there was 94% of sperm with normal morphology and 3% immature and DNA Damage 3% when in *C. sativum* there was 91% of sperm with normal morphology and 7.5% immature and DNA Damage 2.5% and G.a kola and *H. sabdariffa* there were 89% ,79% of sperm with normal morphology and 5.5%,14% immature and DNA Damage 5.5%,7% respectively. This result was the not similar from that obtained by Hee-Sun *et al*., (2013) who reported that in the study of the utility of sperm DNA damage assay using toluidine blue and aniline blue staining in routine semen analysis. The mean age of the study participants was 37.6, 5.0, and the semen samples tested had a mean strict morphology of 5.9%, 3.6%, a mean abnormal sperm structure of 24.1%,14.5%, and a mean abnormal sperm condensation of 18.8%,10.2%.

Conclusion

The results of this study showed that there was decrease sperm DNA damage .Therefore the study concluded that ginger, *Coriandrum sativum, Garcinia kola* and *Hibiscus sabdariffa* possess a protective effect against DNA damage.

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Annexure

Table.1.Phytochemical screening of aqueous extracts of ginger, *Coriandrum sativum, Garcinia kola* and *Hibiscus sabdariffa*

Parameters	Ginger	*Coriandrum sativum*	*Garcinia kola*	*Hibiscus sabdariffa*
Alkaloids	+	_	+	+
Cardiac glycosides	+	+	+	+
Deoxy sugar	_	+	+	+
Flavonoids	+	+	+	+
Phlobatannins	_	_	+	+
Saponins	+	_	+	+
Steroidal ring	+	+	+	_
Tannins	_	+	+	+

+: present, −: absent

Table.2 Comparison effects of ginger, *Coriandrum sativum* ,*Hibiscus sabdariffa* and *Garcinia kola* on sperm DNA damage at dosage 100 mg/kg

Parameter	Morphology%	Immature%	Sperm DNA damage%
Control	85	7	8
Ginger	94	3	3
Coriandrum sativum	90	7.5	2.5
Hibiscus sabdariffa	79	14	7
Garcinia kola	89	5.5	5.5