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Effects of hydro-ethanolic extract of spirulina (*Spirulina platensis*) on semen characteristics and oxidative stress indicators in male rabbit (*Oryctolagus cuniculus*)

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Abstract

Background and purpose: Spirulina is a planktonic blue green alga belonging to the cynobacterium family. It's very rich unusual nutritional profile and the bioavailability of various nutrients make it to be important. This work aimed to evaluate the effects of Hydro-Ethanolic Extract (HEE) of Spirulina platensis on semen characteristics and oxidative stress indicators in rabbit.

Methods: For this purpose, 30 male rabbits were randomly allotted into 5 groups (6 rabbits/group). During 120 days, animals of group 1 orally received 1 ml/kg body weight (bw) of distilled water (control), those of group 2, 100 mg/kg bw of vitamin E, while spirulina-treated rabbits received 100, 200 or 400 mg/kg bw of spirulina extract.

Results: All rabbits (100%) treated with 200 or 400 mg/kg bw of HEE of spirulina showed whitish semen compared to the other groups (80%) which showed 20% of yellowish and 80 % of whitish semen. The percentage of gel presence in the semen decreased in the HEE-treated groups close to control, compared to animals given vitamin E. The individual mobility, the sperm count/ml and per ejaculate increased significantly ($p<0.05$) in rabbits receiving 200 mg/kg bw of HEE compared to control and vitamin E groups. The gross mobility increased insignificantly ($p>0.05$) in males submitted to 400 mg/kg bw of HEE with reference to the control and vitamin E groups. The semen volume declined significantly ($p<0.05$) in HEE-groups compared to distilled water and vitamin E groups. The reverse was observed for the pH. The percentage of spermatozoa having coiled tails, big and small heads, and the total anomalies showed no significant

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(p>0.05) difference among treatments. The activity of superoxide dismutase and the concentration of malondialdehyde decreased significantly (p<0.05) in spirulina extract and vitamin E-treated animals with reference to the control. For total peroxidases, only rabbits submitted to 400 mg/kg bw of spirulina extract registered a significantly (p<0.05) higher value compared to other groups. No significant (p>0.05) difference among treatments was noted for catalase activity.

Conclusion: The administration of HEE of spirulina prevented oxidative stress and improved semen quality in male rabbit.

Introduction

Infertility is one of the major health problems in the life. Approximately 30% of this problem is attributed to male factors [1]. Several conditions can interfere with spermatogenesis and reduce semen quality and quantity, thus lower male fertility. The environmental, chemical and biological impact on mammalian reproduction has been well documented. Indeed, studies conducted by Emtenan et al. [2], Huda et al. [3] and Ngoula et al. [4] showed the harmful effects of high temperatures on reproduction in mammals. In addition, other factors like xenobiotics (heavy metals, pesticides...) have been reported to induce oxidative stress in animals [5-7] and hence involved in many aspects of male infertility, where spermatozoa are rendered dysfunctional by lipid peroxidation and altered membrane function, together with impaired metabolism, morphology, mobility and fertility.

In order to counteract these effects, plants having antioxidant properties such as extracts of green tea [8], aqueous extract of guava leaves [9] and ethanolic extracts of *Bersama engleriana* [10] are being used. Besides, other plants like spirulina also having antioxidant properties have been reported. Nevertheless, studies related to their effects on reproduction are rare.

Spirulina, a planktonic blue green alga, is a traditional food of some Mexican and African people. Increasing interest is being shown in this plant due to its very rich nutritional profile and the bioavailability of various nutrients is very high [11]. Moreover, spirulina exhibit anti-viral, antibacterial, antifungal, antiparasites activities [12] and contains antioxidative pigments [13]. Furthermore, this plant has been shown to be great growth promoters [14, 15]. Despite all these, to the best of our knowledge, studies related to the effects of spirulina on reproduction still remain very scarce. However, regarding its chemical composition and particularly its antioxidant properties, it could fight against oxidative stress and improve male reproduction. Thus, the objective of this work was to evaluate the effects of hydro-ethanolic extract of spirulina on oxidative stress indicators and semen characteristics in male rabbits.

Materials and methods

Experimental zone

The study was conducted at the Teaching and Research Farm (TRF) of the University of Dschang, located in the Sudano-Guinean zone (5°36′ - 5°44′ North Latitude and 9°85′ - 10°06′ East Longitude; altitude 1500 m). The average temperature is 20 °C and the relative humidity is generally greater than 60%. The average annual rainfall is 2000 mm.

Plant material and preparation of hydro-ethanolic extract of Spirulina platensis

Six kilograms (6 kg) of dried granules of spirulina collected from Lake Chad were ground. The obtained powder was macerated in 30 liters of hydro-ethanol at ambient temperature for 72 hours. The mixture was then filtered with the help of the filter paper Whatman number 3. The filtrate was evaporated in a rotary evaporator (60 °C) under reduced pressure, and then went through freeze-drying so as to have a black paste.

Animal material

A total of 30 adult male rabbits (New Zealand breed), aged 4.5-5 months, weighing 2-2.5 kg from the Teaching and Research Farm of the University of Dschang were used. Throughout the experimental period, compounded feed and water were provided ad libitum to animals.

Experimental design

At the beginning of the experiment, animals were weighed and then assigned randomly into 5 groups of 6 rabbits each, comparable in terms of body weight. For 120 days, animals of group 1 (control) daily received orally 1 ml of distilled water per kg of body weight (bw), those of group 2 were given by the same method and the same way as for distilled water, 100 mg/kg bw of vitamin E diluted in water, while the three other groups (3, 4 and 5) were respectively treated with 100, 200 and 400 mg/kg bw of hydro-ethanolic extract of spirulina. The animal body weight was recorded weekly and the doses of solutions adjusted accordingly.

Data collection

Semen collection

Semen was collected using artificial vagina containing warmed water (40-45°C), in animals trained beforehand. Some minutes before collection, an excited doe was placed on the lab table in order to stimulate the male. As soon as the first mount was done, just before intromission, the penis was deviated into the artificial vagina and the sperm was collected in a graduated tube placed at the extremity of the artificial vagina.

Immediately after semen collection, the colour and presence or absence of gel were observed and noted. The volume was evaluated by direct reading from the graduated tube. The pH was done using a pH meter of mark EUTECH Instrument. The viscosity (stickiness) was evaluated by Pasteur pipette.

Microscopic analysis

Spermatozoa mobility, concentration, morphology and plasma membrane integrity. Sperm mobility was assessed by direct examination of 20 µl of semen under light microscope at magnification 400 and the mobility score was attributed according to the method described by Boiti et al. [16]. Semen was diluted 100 times and the sperm cells were counted using the Thoma haemocytometer. The plasma membrane integrity was evaluated using hypo-osmotic swelling test. The sperm morphological anomalies (small and big heads, tails winding) was evaluated using an eosin-nigrosin solution in a total number of 200 spermatozoa [16].

Serum oxidative stress indicators

The determination of malondialdehyde concentration was done by the thiobarbituric acid method (Nilsson et al. [17], while the superoxide dismutase activity was evaluated according to the method decribed by Misra and Fridovich [18]. The catalase (CAT) activity was assessed using the chromic acetate method as described by Sinha [19] and the total peroxydase (PEROX) activity was determined by the potassium iodate method [20].

Statistical analysis

Data were submitted to one-way analysis of variance. When differences were significant between means, they were separated using Duncan post hoc test. The limit of significance was 5%. The analyses were performed using SPSS 20.0.

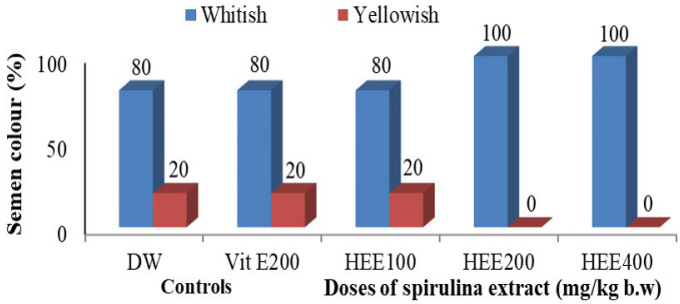

Figure 1: Effects of hydro-ethanolic extract of spirulina on semen colour in rabbit

DW: Distilled Water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: Hydro-Ethanolic Extract of Spirulina at Doses 100, 200 and 400 mg/kg body weight.

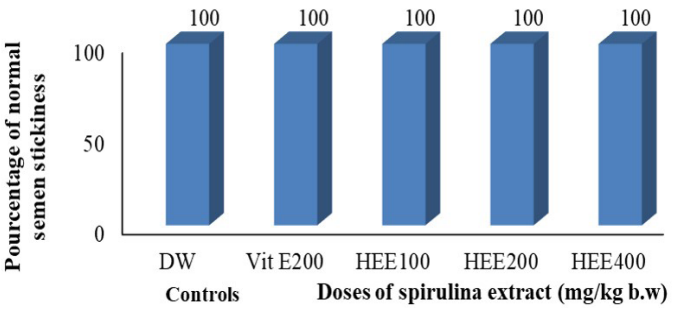

Figure 2: Effects of hydro-ethanolic extract of spirulina on semen stickiness in rabbit

DW: Distilled Water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: Hydro-Ethanolic Extract of Spirulina at Doses 100, 200 and 400 mg/kg body weight.

Results

Semen qualitative characteristics

From the figure 1, it appears that 100% of rabbits in groups treated with 200 or 400 mg/kg of body weight (bw) produced whitish semen, compared to 80% obtained in other groups, where 20% of animals released yellowish semen. The semen stickiness (Figure 2) was normal for all animals no matter the treatments.The gel was present in 60% of semen from the vitamin E-treated group (Figure 3). This percentage decreased in rabbits receiving hydro-ethanolic extract of spirulina, reaching the level of control group at the highest dose (400 mg/kg bw).

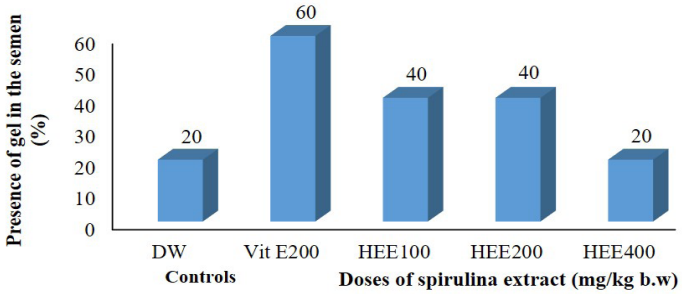

Figure 3: Effects of hydro-ethanolic extract of spirulina on the gel presence in semen in rabbit

DW: Distilled Water; Vit E100: Vitamin E at dose 100 mg/kg body weight; HEE100, 200, 400: Hydro-Ethanolic Extract of Spirulina at Doses 100, 200 and 400 mg/kg body weight.

Semen quantitative characteristics

Table 1 presents the effects of hydro-ethanolic extract of spirulina on semen quantitative characteristics in rabbit. The individual mobility, the sperm count per ml of semen and per ejaculate increased significantly ($p<0.05$) in rabbits receiving 200 mg/kg bw compared to control, vitamin E and other extract-treated groups. The gross mobility increased insignificantly ($p>0.05$) in male submitted to 400 mg/kg bw with reference to those of distilled water and vitamin E groups.

The volume and the percentage of spermatozoa with entire plasma membrane augmented in vitamin E-given rabbits, but the difference was significant ($p<0.05$) only when compared to those treated with the two highest doses of extract for the volume and distilled water, for the spermatozoa plasma membrane integrity.

The percentage of spermatozoa having coiled tails, big and small heads, and the total anomalies showed no significant ($p>0.05$) difference among treatments.

Table 1: Effects of hydro-ethanolic extract of spirulina on semen quantitative characteristics in rabbit

Semen characteristics	Controls		Doses of spirulina extract (mg/kg b.w)			p
	DW (n=6)	Vit E100 (n=6)	100 (n=6)	200 (n=6)	400 (n=6)	
Gross mobility (%)	74.00±8.94^{ab}	76.00±8.94^{ab}	70.00±10.00^{b}	76.00±5.48^{ab}	82.00±4.47^{a}	0.04
Individual mobility (%)	70.00±10.00^{b}	70.00±10.00^{b}	84.00±11.40^{ab}	90.00±7.07^{a}	72.00±10.00^{b}	0.01
Conc/ml (x10^{6})	332.00±0.44^{ab}	239.20±0.45^{b}	276.99±0.73^{b}	420.00±0.73^{a}	300.20±0.90^{b}	0.01
Conc/ejaculate (x10^{6})	657.60±0.91^{b}	389.96±0.84^{d}	479.28±0.74^{cd}	867.84±1.28^{a}	526.40±0.84^{c}	0.00

Volume (ml)	1.68±0.24^{a}	1.90±0.41^{a}	1.56±0.30^{ab}	1.34±0.27^{b}	1.40±0.38^{b}	0.04
pH	6.78±0.18^{ab}	6.56±0.13^{b}	6.86±0.22^{a}	6.90±0.22^{a}	6.74±0.13^{ab}	0.04
Membrane integrity (%)	83.60±5.59^{b}	92.60±3.85^{a}	85.00±5.61^{ab}	87.00±6.63^{ab}	88.00±6.96^{ab}	0.04
Big and small head spermatozoa (%)	1.10±0.55	0.70±0.27	1.10±0.42	1.60±0.42	1.40±0.42	0.09
Coiled tail spermatozoa (%)	4.20±1.15	4.50±1.54	4.40±1.52	3.60±1.29	3.10±0.82	0.40
Total anomalies (%)	5.30±1.10	5.20±1.52	5.50±1.77	5.20±1.44	4.50±0.94	0.83

a, b, c, d: on the same ligne, values affected with the same letter are not significantly different (p>0.05). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; Conc: sperm concentration (sperm count).

Oxidative stress markers

Table 2 resumes the effects of hydro-ethanolic extract of spirulina on some oxidative stress markers in rabbit. Generally, the activity of superoxide dismutase and the concentration of malondialdehyde decreased significantly (p<0.05) in spirulina extract and vitamin E-treated animals with reference to the control receiving distilled water. For the total peroxidases, only rabbits submitted to 400 mg/kg bw of spirulina extract registered a value significantly (p<0.05) higher compared to other groups. The catalase activity showed no significant (p>0.05) difference among treatments. The serum content in all evaluated stress markers recorded in rabbits exposed to hydro-ethanolic extract of spirulina were comparable to that of vitamin E-treated animals.

Table 2: Effects of hydro-ethanolic extract of spirulina on some oxidative stress markers in rabbit

Oxidative stress markers	Controls	Doses of spirulina extract (mg/kg b.w)				p
	DW (n=6)	Vit E100 (n=6)	100 (n=6)	200 (n=6)	400 (n=6)	
SOD (U/min/mg of testicular protein)	21.01±2.90^{a}	17.02±3.12^{ab}	15.09±1.17^{b}	18.19±3.91^{ab}	14.16±1.39^{b}	0.02
CAT (µM/min/g of testis)	14.12±1.91	11.64±1.20	13.32±2.33	12.96±2.47	13.15±2.35	0.52
Total peroxidases (mM/min/g of testis)	0.39±0.08^{b}	0.40±0.09^{b}	0.34±0.07^{b}	0.42±0.07^{b}	0.53±0.09^{a}	0.01
MDA (µM/g of testis)	0.94±0.04^{a}	0.78±0.07^{b}	0.83±0.05^{b}	0.82±0.06^{b}	0.87±0.07^{b}	0.02

a, b: on the same ligne, values affected with the same letter are not significantly different (p>0.05). n: number of animals. DW: distilled water; Vit E100: Vitamin E at dose 100 mg/kg body weight; SOD: superoxide dismutase; CAT: catalase; MDA: malondialdehyde.

Discussion

The use of plant extracts as fertility enhancer in animals is now on the increase because of the shifting attention from synthetic drugs to natural plant products [21]. Natural plants such as spirulina contain bioactive molecules including phenols, terpenes, alkaloids and others. These molecules possess properties (antioxidants, antibacterial, anti-inflammatory, antiseptic, anti-parasitic and immunomodulatory) which can be used in male animals to reduce the spermatozoa characteristic impairment and subsequently increase the fertility traits [22]. In the present study, all rabbits treated with hydro-ethanolic extract of spirulina showed whitish semen colour. In the same line the semen stickiness was normal for all treatment and the percentage of gel decreased in the hydro-ethanolic extract-treated groups close to control. This could be due to the androgenic activities of bioactive compounds found in this extract which had an effect on the accessory sex glands, since they are highly responsible for the constitution of semen. The semen characteristics (colour, stickiness and percentage of gel) in male animals could be used to predict the animal fertility capacity. The stickiness and colour respectively bring out the idea on the spermatozoa density and the metabolism that takes in the sperm. In the other hand, the percentage of gel would negatively correlate to spermatozoa mobility. Its increase in the sperm would linked to the decrease in spermatozoa movement. The individual and gross mobility, the sperm count/ml of semen and per ejaculate increased in rabbits receiving hydro-ethanolic extract of spirulina. The volume decreased and the percentage of spermatozoa with entire plasma membrane increased in the hydro-ethanolic extract-groups with reference to the control. These results are similar to the findings obtained by Rudic et al. [23] in bull treated with spirulina. Also, Akinola et al. [24] and Uboh et al. [25] showed that the aqueous extract of Psidium guajava caused a significant increase in the mobility of spermatozoa. The synergetic effects of antioxidative and androgenic compounds revealed in spirulina might have stimulated and improved the semen production and quality. The spermatozoa are particularly rich in polyunsaturated fatty acid which predisposes them to lipid peroxidation by reactive oxygen species, associated with spermatozoa membrane and DNA impairment. The spirulina bioactive molecules with antioxidant properties would protect the spermatozoa from the reactive oxygen species attack. This effect consequently increases the thickness of the membrane, the mobility and the sperm count. According to Tchoffo et al. [22], substances with antioxidant properties could be used in animal production to reduce the cell membrane free radical attack, resulting in an improvement in germ cell characteristics. In the present study, the concentration of MDA and activities of SOD and CAT reduced in extract-treated rabbits compared to the control. These results corroborate the findings of Romay et al. [26]; Bhat and Madyastha [27] and Pinero Estrada et al. [28] on extracts of Spirulina platensis. This could be due to the phenolic compounds present in hydro-ethanolic extract of spirulina. Indeed, Jaime et al. [29]; Plaza et al. [30] and Guan et al. [31] reported that phenolic compounds are responsible for the antioxidant activities of many plants. These properties protect animal cells from the harmful effects the reactive oxygen spe-

cies and reduce the blood content in MDA which is the resulting product of lipid peroxidation. Since spermatozoa are highly rich in fatty acids, the presence of antioxidant molecules in the extract could have inhibit the lipid peroxidation, therefore, improving the semen quality.

Conclusion

Hydro-ethanolic extract of spirulina due to their various micronutrients and pharmacological active compounds (phenols, terpenes, alkaloids) improves the spermatozoa characteristics and subsequently the fertility traits.

Ethical issue

Experimental protocol used in the present study was in accordance with recommendations of institutional guidelines for the care and use of laboratory animals. rabbits were humanly handled in respect of the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

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