ORIGINAL ARTICLE

Sperm function and fertility profile following nicotine administration in male rats: Protective potentials *of Zingiber officinale*

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Background: It is well documented that *Zingiber officinale* has androgenic property while nicotine induce infertility through hormonal imbalances, but there are no data in the open scientific literature that have examined the effects of *Z. officinale* in preventing nicotine-induced infertility. This study was carried out to investigate the effect of *Z. officinale* on nicotine-induced reproductive toxicity in male rats. **Materials and Methods:** Thirty-two male albino rats were randomly divided into four groups (n = 8). Group 1 served as the control, group 2 was administered with 1.0 mg/kg body weight (BW) of nicotine orally for 30 days, group 3 was administered with 1.0 mg/kg BW of nicotine alongside with 500 mg/kg BW/day of aqueous extract of *Z. officinale* and group 4 was administered with 500 mg/kg BW/day of aqueous extract of *Z. officinale*. Semen analysis showing sperm count, morphology, motility, volume and viability was carried out. Fertility study, litter weight and size were also determined. Testosterone was also assayed. **Results:** Nicotine treatment significantly decreased sperm count, motility, normal morphology and serum testosterone level. There was a decrease in libido, litter weight and number delivered by the untreated cohabited female during the experiments. However, the extract prevented the decrease in sperm quality and hormonal imbalances caused by nicotine. **Conclusion:** This finding shows that aqueous extract of *Z. officinale* prevented nicotine-induced infertility during nicotine administration and possesses pro-fertility potentials attributed to the androgenic properties of the plant.

Key words: Hormone, nicotine, sperm, *Zingiber officinale*

INTRODUCTION

Zingiber officinale commonly called ginger is used as food spices in African and Asian countries. This plant has been documented to contain compounds such as folic acid, inositol, choline acid resins, panthotenic acid sesquiterpene, gingerol, vitamin B3 and B6, volatile oils and some bio-trace element.[1] Ginger has been shown to possess medicinal value in literature, it has been reported to have protected against sodium-arsenite-induced reproductive toxicity and oxidative stress. This is attributed to its antioxidant and androgenic properties.[2] Katmchouing *et al.*, has also reported the androgenic activities of ginger in male rats[3] while studies have shown that it ameliorated hepatotoxicity and cardiotoxicity induced by cisplatin in rats.[4]

Nicotine is considered as the primary chemical in tobacco that is responsible for enhancing, tobacco use and dependence.[5,6] This might be the reason why cigarette smoking is still very common despite the worldwide anti-smoking campaigns. Nicotine can be consumed in different forms ranging from smokeless tobacco product such as snuff and chewing tobacco but more often consumed as smoked tobacco. It constitutes 90-95% of the total alkaloids,[7] which is absorbed quickly through the respiratory tract, oral muscosa and skin. Approximately 80-90% of nicotine is metabolised by liver, however, the kidney and lungs are involved as well.[8]

Several adverse reproductive effect of nicotine has been documented in literature. Recently, it was documented that nicotine decrease reproductive organ weight, causes testicular degeneration, disorganisation of the cytoarchitective and also decrease serum testosterone level,[9] although this effect was ameliorated upon cessation. A high dose treatment of nicotine has also been shown to have deleterious effect on sperm characteristics and fertility index; however, these effects were also ameliorated by nicotine cessation in male albino rat.[10]

In spite of the growing knowledge of adverse reproductive effects of nicotine on reproduction through smoking and the beneficial potentials of *Z. officinale*,

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.129587

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Received: 02-11-2013; **Accepted:** 11-01-2014

it is relatively unknown whether or not *Z. officinale* could ameliorate nicotine-induced infertility. However, no study has documented the effect of this medicinal plant on nicotine-induced infertility despite its androgenic property. The present study was therefore designed to investigate the effect of co-administration of *Z. officinale* on nicotine-induced reproductive toxicity.

MATERIALS AND METHODS

Animals and Treatment

Nicotine Preparation

Nicotine hydrogen tartrate with product number 26140 (95% nicotine) was purchased from BDH chemical Ltd., Poole, England. The nicotine dosage freshly prepared in normal saline for each group of animals was delivered orally at 1.0 mg/kg body weight (BW). The working solutions were stored in foil-wrapped glass bottle at 4°C for no longer than 10 days.

Animals and Treatments

Experiments were performed on 32 male albino rats whose average weight ranged between 150 and 180 g (8-10 weeks old) obtained from the Animal House, College of Medicine, University of Ibadan, Oyo State, Nigeria. Animals were divided into four equal groups with *ad libitum* access to rat chow and drinking water. Animals were also maintained in a well-ventilated room with a 12:12-hour light–dark at room temperature. The experiment was conducted in accordance with the Guidelines of the US National Institute of Health (NIH) on the care and use of laboratory animals. The animals were divided into four groups; Control group that received 0.2 ml/kg normal saline, group 2 was treated with 1.0 mg/kg nicotine, Group 3 was treated with 1.0 mg/kg nicotine alongside with 500 mg/kg BW/day of aqueous extract of *Z. officinale* (AZO) and group 4 was administered with 500 mg/kg BW/day of AZO.

Extract Preparation

AZO was purchased from local commercial sources in Osogbo, Osun State, Nigeria and prepared following the procedures of Morakinyo *et al*.[2] Briefly, dried *Z. officinale* rhizomes were pulverised and soaked in distilled water for 48 h. A final AZO concentration of 100 mg/ml was obtained following maceration method. This stock solution was prepared on weekly basis following the same procedure and kept at 4°C to maintain its potency.

Semen Collection

The left testis was removed along with its epididymis. The caudal part of the epididymis was separated from the testis and lacerated to collect the semen on a microscope slide for semen characteristics evaluation as previously described.[11]

Sperm Characteristics Analysis

Progressive motility of sperm was tested immediately. Semen was squeezed on the pre-warmed slide and then two drops of warm 2.9% sodium citrate was added to it. This was covered with a cover slip and then examined and scored under the microscope using ×40 objective with reduced light.[12] A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. Semen was squeezed onto a microscope slide and few drops of the stain were added. The motile sperm cells were unstained while the non-motile sperm absorbed the stain. The stained and the unstained sperm cells were counted using ×40 objectives of the microscope and an average for each was taken from which percentage viability was calculated. Sperm morphology was done by staining the sperm smears on the microscope slides with two drops of Walls and Ewas stain. This was then air-dried. The slides were examined under the microscope using ×100 objectives under the oil immersion. The abnormal sperm cells were counted and the percentage calculated according to the method described by Wyrobek and Bruce.[13] To evaluate the sperm volume, the epididymis was immersed in 5 mL normal saline in a measuring cylinder and the volume displaced was taken as the volume of the epididymis. Sperm count was done under a microscope with the aid of the improved Neubauer hemocytometer.[14]

Libido Test

To observe the libido-oriented mounting behaviour of male rat, non-oestrous untreated female rats were paired on the 30th day at 6.00 pm. The male rats assuming the copulatory position over the female, but fail to achieve intromission was considered as a mount.[15] Male rats from each group were chosen and suitably marked. The rats were placed in a clear aquarium, allowed to acclimatise for 15 min after which a non-oestrous female was introduced into the arena. The number of mount was recorded for 15 min for each animal. This process was also done for the experimental groups.

Fertility Studies

A total of 20 untreated fertile, Proestrum female rats were used for the fertility test. Five untreated female rats were cohabited with each other of the four male groups from the day 31 of treatment. All animals were cohabited for 5 days according to earlier studies.[15] The presence of a vaginal plug was accepted as the index for a positive mate and taken as day one of pregnancy.[16] A fertility test was calculated using this formula.[17]

$$\% \text{ Fertility success} = \frac{\text{Pregnancy female}}{\text{Mated female}} \times 100$$

The number of litters delivered and their body weights were determined.

Testosterone Assay

An enzyme-based immunoassay (EIA) system was employed to determine testosterone in the plasma samples collected as described in earlier studies.[9] The reagent was obtained from immunometrics (London, UK).

Statistical Analysis

Data obtained were expressed in Mean ± SEM. Statistical analysis was performed by analysis of variance (ANOVA) followed by multiple comparison by two-tailed *t*-test. The values for $P < 0.05$ were considered to be statistically significant.

RESULTS

Effect of Nicotine and AZO on Motility

Oral administration of 1.0 mg/kg BW of nicotine on animals daily for a period of 4 weeks significantly decrease ($P < 0.05$) the progressive motility of the sperm when compared with the control group. Animals co-treated with 1.0 mg/kg nicotine treated and AZO had an insignificant decrease ($P > 0.05$), whereas AZO only treated group had a significant increase ($P > 0.05$) in sperm motility when compared with the control as shown in Table 1.

Effect of Nicotine and AZO on Epididymal Sperm Count

The mean epididymal sperm count of animals administered with 1.0 mg/kg BW was significantly decreased ($P < 0.05$) when compared with their control. However, animals co-treated with 1.0 mg/kg nicotine and AZO had comparable values with the control. A significant increase in sperm count was observed in AZO alone groups when compared with the control and nicotine-treated rats as shown in Table 1.

Effect of Nicotine and AZO on Viability (Live/Dead Ratio)

A significant decrease ($P > 0.05$) was recorded for the mean percentage live sperm of rats treated with 1.0 mg/kg BW when compared with the control. However, animals co-treated with 1.0 mg/kg nicotine and AZO and AZO only treated groups had values that were not statistically different from the control as shown in Table 1.

Effect of Nicotine and AZO on Epididymal Sperm Volume

The result showed that nicotine caused an insignificant decrease ($P > 0.05$) in the epididymal sperm volume in the treated groups when compared with the control as shown in Table 1.

Effect of Nicotine and AZO on Morphology

Administration of 1.0 mg/kg BW nicotine significantly reduced normal sperm morphology. Co-treatment with AZO abolished this effect. There was no significant difference in the sperm morphology of animals treated with AZO only and the control group as shown in Figure 1. However, the most predominant abnormality observed during the morphological examination of the sperms in the animals treated with nicotine daily was the "curve tail". Although there seems to be fewer occurrence of the 'curve tail' morphological aberration in other experimental groups as recorded in Table 2.

Effect of Nicotine and AZO on Fertility Index

Administration of 1.0 mg/kg BW nicotine caused significant decrease in libido score when compared with the control. Administration of AZO prevented libido score decrease as observed in rats co-treated with AZO. Similarly, rats treated with AZO only had comparable libido score with those of the control and co-treated AZO groups as shown in Table 3.

Table 1: Semen parameters of experimental rats treated with nicotine and AZO

Dose	Motility (%)	Live/dead ratio (%)	Volume (ml)	Count (10^6/ml)
Control	85.50±3.21	94.56±4.21	5.22±0.04	112.40±10.40
1.0 mg/kg+ Nicotine	32.00±4.65*	79.80±5.17*	5.18±0.06	64.20±5.60*
1.0 mg/kg+ AZO	83.80±5.01	90.60±3.38	5.22±0.05	108.3±8.14
AZO	96.00±6.52*	93.20±6.38	5.20±0.02	128.60±6.80*

AZO – Aqueous extract of *Z. officinale*. Values are expressed as means ± S.E.M of 8 rats per group. * $P<0.05$ vs control

Table 2: Sperm abnormalities of experimental rats treated with nicotine and AZO

Sperm parameters	Control	0mg/kg BW	1.0 mg/kg nicotine+AZO	AZO
Tailless head	3	4	5	2
Headless sperm	4	6	6	0
Rudimentary tail	2	11	1	3
Curved tail	9	39	12	6
Curved midpiece	4	6	5	3
Bend midpiece	5	7	4	4
Coiled-tail	2	3	3	1
Swapped-tail	1	2	2	0
Total number of abnormal sperm	30	78	38	18
Total number of normal sperm	370	322	362	382
% of Abnormal cells	8.10	24.22*	10.50	4.71*

AZO – Aqueous extract of *Z. officinale*. Values are expressed as Means ± S.E.M of 8 rats per group. Means in rows not sharing common superscript letters are not significantly different; $P<0.05$

Table 3: Fertility profile of experimental rats treated with nicotine and AZO

Dose	Libido score	Litter number	Litter weight (g)	Percentage fertility
Control	8.82±1.21	6.24±0.40	5.96±0.20	100
1.0 mg/kg+ Nicotine	3.26±1.62*	0.00±0.00*	0.00±0.00*	0*
1.0 mg/kg nicotine+AZO	8.64±2.02	6.56±0.50	5.74±0.28	90
AZO	9.45±2.41	9.33±0.54*	7.82±1.10*	100

AZO – Aqueous extract of *Z. officinale*. Values are expressed as means ± S.E.M of 8 rats per group. *$P < 0.05$ vs control

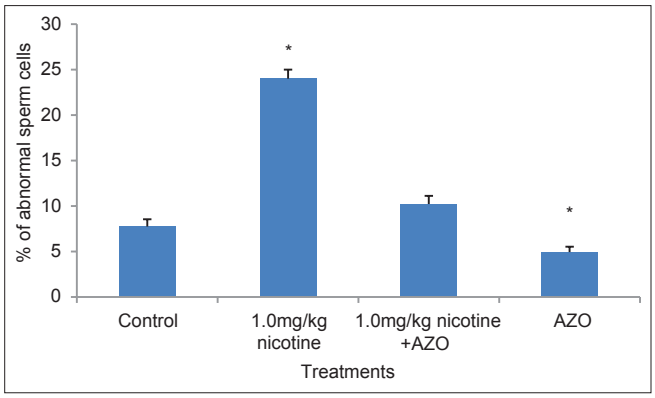

Figure 1: Effect of nicotine and aqueous *Zingiber Officinale* (AZO) on mean percentage abnormal sperm cells in male rats. Values are expressed as means ± S.E.M of 8 rats per group. Bar carrying the asterisk sign on each parameter are significantly different from control at $P < 0.05$

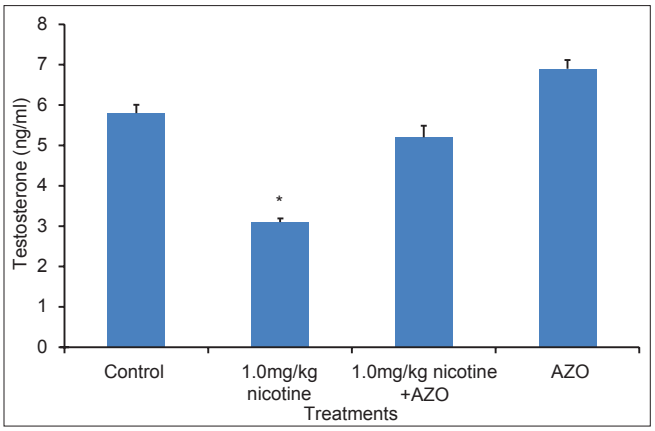

Figure 2: Effect of nicotine and aqueous *Zingiber Officinale* (AZO) on serum testosterone level in male rats. Values are expressed as means ± S.E.M of 8 rats per group. Bar carrying the asterisk sign on each parameter are significantly different at $P < 0.05$

Table 3 shows that nicotine significantly decreased percentage fertility when compared with the control. Co-administration of AZO prevented nicotine induced-infertility. Similarly, rats treated with AZO only had comparable percentage fertility with those of the control and co-treated AZO groups. In addition, a significant increase in weight and number of pups was observed in the AZO only treated group when compared with the control as shown in Table 3.

Effect of Nicotine and AZO on Serum Level of Testosterone

The mean serum testosterone level of animals that received 1.0 mg/kg BW of nicotine was significantly decreased ($P < 0.05$) when compared with the control group. However, nicotine groups co-treated with AZO and AZO only groups had comparable values with the control, although an insignificant increase in testosterone level was observed in AZO only groups when compared with the control as shown in Figure 2.

DISCUSSION

The results of this present study revealed that AZO has protective effect on reproductive dysfunction of nicotine in male rat. To our knowledge, this is the first study that evaluated the protective effect *Z. officinale* against reduced sperm function and fertility profile induced by nicotine in experimental animals. The decrease sperm count, motility and normal sperm morphology of rats observed in this study agrees with the previous report, which demonstrates that nicotine impair sperm qualities.[10] The significant reduction in sperm count, motility and morphology observed may be associated with an impartment of spermatogenesis consequent to reduction of testosterone secretion caused by nicotine.[9] This also agrees with a previous report on cigarette smoking-induced impairment of spermatogenesis documented in previous studies.[18] Co-administration of nicotine and AZO clearly ameliorated nicotine-induced infertility and maintained normal sperm function and fertility. This may be due to its androgenic activities since testosterone was significantly increased with AZO extract administration.

Sperm count, motility and morphology are key indices of male fertility as these are prime markers of testicular spermatogenesis.[2] Low testosterone concentration may be responsible for the adverse effect of nicotine on sperm parameter as high level of testosterone is critical for normal spermatogenesis, development and maintenance of sperm morphology.[19] The maintenance of normal sperm parameters by AZO may result from its ability to maintain high level of testosterone since it was observed that there were high levels of serum testosterone in nicotine group co-administered with AZO and the AZO treated group only.

Fertility studies showed a significant decrease in libido score of animals treated with nicotine. This is also probably associated with the decrease in serum testosterone level observed because testosterone has been associated with increased sexual, physical and mental energy, stamina and vitality and sexual drive.[20] The decreased average litter number delivered by the untreated females cohabited with nicotine treated male rats might be the effects of nicotine on progressive sperm motility while prevention of decrease litter number could be attributed to the ability of the extract to maintain and improve sperm motility in experimental animals.

CONCLUSION

This reduction in sperm function and fertility rate was prevented by *AZO* administration, which could be attributed to its ability to maintain sperm parameter integrity and testosterone level.

The restoration of testosterone level to normal with co-administration with AZO might have stimulated the

production of qualitatively and structurally normal sperm through the plant's androgenic properties, thus establishing a mechanism by which the extract prevents nicotine-induced damage to sperm function and fertility indices.

ACKNOWLEDGMENT

This research work was sponsored by the Tertiary Education Trust fund (TETfund) of Nigeria.

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How to cite this article: Oyeyipo IP, Obembe OO, Oladokun OO, Raji Y. Sperm function and fertility profile following nicotine administration in male rats: Protective potentials of *Zingiber officinale*. Int J Green Pharm 2014;8:125-9.

Source of Support: Tertiary Education Trust fund (TETfund) of Nigeria, **Conflict of Interest:** None declared.