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Ethanol extract of the leaves of *Psidium Guajava Linn* enhances sperm output in healthy wistar rats

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Summary

Reactive oxygen species (ROS), among other factors, have been implicated in the aetiology of male infertility. Thus, the roles of antioxidants at improving sperm production and quality are being investigated. The present study was designed to assess the effect of the ethanol extract of fresh leaves of *Psidium guajava Linn.* on the sperm parameters of healthy male Wistar rats. A total of 18 rats, weighing between 108-124 g, were divided into 3 groups of 6 animals each. Animals in groups 1 and 2 were administered 250 mg/kg/d and 500 mg/kg/d of guava leaf extract (GLE) orally for 53 days respectively. Group 3 animals received normal saline. Sperm count increased from 56.2±0.3 ($X10^6$) in the control to 57.1±0.2 ($X10^6$) in group 1 animals, and from 56.2±0.3 ($X10^6$) in the control to 72.3±0.4 ($X10^6$) in group 2 animals. Similarly, dose-dependent increases in the percentages of motile spermatozoa were observed in GLE-treated animals compared to the control group. These findings suggest that the extracts of the leaves of *Psidium guajava Linn.* possess beneficial effects on sperm production and quality, and may thus improve the sperm parameters of infertile males with oligospermia and non-obstructive azoospermia.

Keywords: *Psidium guajava, antioxidants, male infertility, spermatozoa*

Résumé

Cette étude était faite pour évaluer les effets des extraits des feuilles fraîche des *Psiduim guajava Linn* sur les paramètres des spermes aux rats males sain ;18rats entre 108-124g étaient divisé en 3 groupes de 6 rats chacun. Les animaux du groupe de 1 et 2 recevaient oralement de 250 mg /kg /day d'extraits des feuilles de guave pour 53 jours. Les animaux du groupe 3 recevaient l'eau salée normale uniquement. Le taux des spermes augmentait de56.2 ± 0.3 ($x10^6$) au groupe contrôle à 57.1 ± 0.2($x10^6$) mg/kg/day au groupe 1 et 72.3 ± 0.4($x10^6$) aux animaux du groupe 3. Semblablement, l'augmentation des extraits était dose dépendent dans le pourcentage des spermes mobile et étaient élevés aux groupes recevant les extraits des feuilles de guave comparés aux contrôles. Ces résultats suggèrent que les extraits des feuilles de *Psiduim J. Linn* possèdent des effets bénéfiques sur la production et la qualité des spermes, et peut améliorer les paramètres d'infertilité des hommes ayant l'oligospermie et l'azoospermie non obstructive.

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Introduction

Reactive oxygen species (ROS) have been implicated in infertile men with oligospermia, azoospermia and/or structural and genetic aberrations of the spermatozoa [1, 2]. ROS include superoxide, nitric oxide, hydroxyl radical, hydrogen peroxide, and peroxynitrite. In males, the levels of ROS increase to toxic quantities in the presence of high quantities of immature and abnormal spermatozoa, contamination with leucocytes, sperm processing such as excessive centrifugation, and low antioxidant levels in the seminal plasma [3].

When present in high quantities, ROS impair sperm motility, sperm survival and function by interacting with membrane lipids, proteins, and nuclear and mitochondrial DNA [4, 5]. Mammalian spermatozoa are especially susceptible to oxidative insult because their membranes are rich in polyunsaturated fatty acids that enhance the fluidity of these membranes [6]. Besides, the cytoplasm of spermatozoa contains low concentrations of reactive scavenging enzymes [7]; thus, spermatozoa depend on the protection afforded by antioxidants in the seminal plasma [8].

There are evidences that antioxidants could protect spermatozoa against oxidants, and thus possibly enhance male fertility, among other potential

benefits [9, 10, 11]. Thus, the objective of this study was to test the effect of the ethanol extract of fresh leaves of *Psidium Guajava Linn.* (guava) on the sperm parameters of healthy Wistar rats.

Psidium Guajava Linn. is a common tropical plant with a spectrum of bioactive phytochemicals, which include certain phenolic compounds (tannins, quercetin, avicularin, and guaijaverin), carotenoids and ascorbic acid [12, 13, 14]. These phytochemicals possess demonstratable radical-scavenging activity [15], and may therefore be beneficial to males with oligospermia and non-obstructive azoospermia induced by oxidative stress.

Methods

Preparation of Ethanol extract of the leaves of Psidium Guajava Linn

Fresh leaves of *Psidium guajava Linn.* were collected and authenticated at the Department of Crop Production, Faculty of Agriculture, University of Ilorin. 200 g of the leaves was pounded in porcelain mortal, and placed in a beaker; 400 ml of 50 % ethyl alcohol was then added, and left for 30 hours. Thereafter, the mixture was sieved and filtered with Whitman filter paper. The resulting extract was concentrated to 10 % of its initial volume using the water bath at 40 °C, in a closed environment.

Animals and treatment

Eighteen adult (12-16 weeks old) male Wistar rats, weighing between 108-124 g were procured from the Department of Biochemistry, University of Ilorin. All animals were housed in the Laboratory for Reproductive Biology, Department of Anatomy, University of Ilorin. They were exposed to 12 hours light:12 hours dark photoperiod. Standard rat chows (Shepherd Touch Mill, Ilorin) and water were given *ad libitum*. The animals were sorted randomly into three groups of six animals each. These groups were treated orally with guava leaf extract (GLE) as follows:

Group 1: 250 mg/kg/d of GLE
Group 2: 500 mg/kg/d of GLE
Group 3: (Control): Normal saline

At the end of the 53-day treatment period, all rats were anaesthetized with 60 mg/kg pentobarbital sodium intraperitoneally. The caudal epididymis of each animal was dissected, excised, minced and placed in 1 ml of Ham's F 10 solution.

Sperm count and motility

0.5 ml of sperm/Ham's F10 solution was drawn into a white cell pipette and placed in the Neubauer counting chamber of a haemocytometer for the evaluation of sperm count. Sperm motility was assessed with the light microscope.

Statistical analysis

Data collected for sperm count and motility were analysed using the student's t-test. $P<0.05$ was considered significant.

Results

As indicated in table 1, ethanol extract of *Psidium guajava Linn* produced dose-dependent increases in sperm count in animal groups 1 and 2. In group 2 animals, the increase in sperm count was statistically significant ($P<0.05$). Similarly, GLE increased the motility of the spermatozoa as suggested by the increases in the percentage of motile spermatozoa in groups 1 and 2. The percentages of motile spermatozoa increased ($P>0.05$) from 65.3 % in the control group to 66.2 % in group 1 animals, and from 65.3 % in the control group to 81.1% in group 2 animals ($P<0.05$)

Table 1: Effect of *Psidium guajava Linn* on sperm count and sperm motility of Wistar rats

Group of Animals	Sperm count (x10⁶/ml)	Sperm motility (%)
1 (250 mg/kg/d GLE)	57.1±0.2	66.0±3.4
2 (500 mg/kg/d GLE)	*72.3±0.4	*81.1±2.4
3 (Normal saline)	56.2±0.3	65.3±2.1

Data expressed as Mean±SEM
**Significantly different from control. P<0.05*

Discussion

Defective sperm function could arise from oxidative stress created by excessive generation of reactive oxygen species (ROS) by spermatozoa, and/or disruption of the antioxidant defence system in the male reproductive tract [16, 17]. The presence of large quantities of polyunsaturated fatty acids in the plasma membranes of spermatozoa [18], and the low concentrations of scavenging enzymes in the cytoplasm of these cells [16, 19] could account for their susceptibility to oxidative stress. ROS do not

only have adverse effects on the fluidity of the plasma membrane of spermatozoa, but also on the integrity of the nuclear DNA of these cells [20]. Thus, maintenance of an optimal level of ROS is required for the regulation of sperm production and function [21].

Experimental findings suggest that low concentrations of these radicals are beneficial to spermatogenesis [22], and sperm motility [23, 24, 25]. Conversely, high concentrations of ROS inhibit the motility of mouse [26], and human [27] spermatozoa, as well as impair sperm viability [28]. These effects arise probably from the cytotoxic effect of reactive species, mediated by oxidative stress and lipid peroxidation of sperm plasma membranes [17]. Thus, a battery of different antioxidants is needed to protect sperm against oxidative damage [29].

The present study therefore investigated the effects of ethanol extract of *Psidium guajava Linn.* on the sperm parameters of Wistar rats. As indicated in table 1, sperm count and motility increased in a dose-dependent manner following the exposure of the experimental rats to guava leave extract for 53 days. These findings could be owing to the ability of the phytochemical antioxidants in the administered extract to adjust the concentrations of ROS in the testes and genital tracts of the animals to levels that are optimal for sperm production and maturation. Previous studies showed that the leaves of *Psidium guajava* possess strong free radical-scavenging activity [30, 31, 32]

In related studies, the effects of the vitamin antioxidant, á tocopherol, was assessed on free radical-induced age-related changes in the epididymides of brown Norway rats. Vitamin E supplementation was reported to decrease oxidative stress-related changes in epididymal structure. Besides, daily oral administration of trans-resveratrol, a natural antioxidant in grapes, to healthy rats was reported to produce significant increases in sperm count [9]. These, and other related findings [10, 33] are suggestive of the beneficial effects of natural antioxidants against oxidative stress and the disease conditions induced by ROS.

Conclusion

Our findings in the present study in rats suggest that leaf extracts of *Psidium guajava Linn.* could be beneficial to males with oligospermia and non-obstructive azoospermia that are consequent upon oxidative insult. Thus, the roles of the phytochemical antioxidants in this herb at enhancing the fertility status of male individuals could be further explored.

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