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Effect of Guava (*Psidium guajava*) Leaves Essential Oil on Some Reproductive Parameters in Male Guinea Pig (*Cavia porcellus*)

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Abstract

Aim: To assess the effects of essential oïl from guava leaves on some reproductive parameters.

Methods: Thirty-two male guinea pigs (Cavia porcelus) of three months old and weighing between 350 and 400 g were used. These guinea pigs were divided into 4 groups of 8 animals each. Each group was randomly attributed orally for 60 days one of the following guava leaf essential oil doses: 0 (control group), 80, 100 and 120 µl of oil per kg body weight. At the end of treatment, all animals were sacrificed to assess the reproductive parameters.

Results: Body weight, relative weight of the seminal vesicles, vas deferens, and epididymis were not significantly increased ($p>0.05$) with the considered guava leaves essential oil doses. The testes weights significantly increased ($p<0.05$) in animals who received 100 and 120 µl/kg body weight compared to control. A significant increase ($p<0.05$) in sperm concentration, mobility and viability was observed in animals which received the essential oil doses compared to control. Also, the level of proteins and testosterone in serum and testes significantly increased ($p<0.05$) with the essential oil doses.

Conclusion: Guava leaves essential oil can be used to improve male reproductive performances.

Keywords: Essential oil; Guava leaves; Male reproductive system; Guinea pig

Introduction

Essential oil (or sometimes vegetable gas) is the concentrate and hydrophobic liquid of volatile aromatic compounds (odoriferous) of a plant. It is specific to seed coniferous plants and is found in the families of Labiatae and Myrtaceae [1]. Although the essential oils have some common points in their modes of action, their properties are varied: they are anti-infectious, antifungal, antiparasitic, antibacterial, anti-inflammatory, etc. These properties are used in animal production [1,2].

Guava (*Psidium guajava*) is a fruit tree of the family of Myrtaceae, native from South America and growing in the tropics. All parts (roots, fruits, leaves, etc.) are used in traditional medicine for the treatment of diseases such as gastrointestinal and respiratory disorders, diabetes, cancer, etc. [3-5]. Studies on extracts from the leaves of *P. guajava* and essential oil they contain showed that they possess anti-inflammatory, antibiotics, analgesics, hepato protective and antioxidant activities [6-8].

Many studies have been conducted in the field of animal production; on the effects of essential oils on growth performance [9-12], but very little information exist regarding their effects on reproductive performances. Experimental studies showed that in rats treated with leaves extracts from *Psidium guajava*, there was an increase in the levels of reproductive hormones (testosterone, LH, FSH), of testes and epididymis weight, of mobility and epididymal sperm concentration [13-16]. Although these studies showed some positive effects of guava leaves extracts on reproductive performances, no studies on the effects of its essential oil on these parameters have been performed. Thus the main objective of this work was to determine the effects of guava leaves essential oil on some reproduction parameters in male guinea pig.

Materials and Methods

Plant material and essential oil extraction

Fresh guava leaves were collected from the Dschang University campus, dried in the shade and then crushed. Oil extraction was done by hydrodistillation in the Laboratory of Animal Physiology of the Faculty of Agronomy and Agricultural Sciences. After separation, the oil was filtered through anhydrous sodium sulphate to remove any trace of water.

Animal material

Thirty-two (32) males guinea pigs (*Cavia porcellus*) aged 3 months old and weighing between 350 and 400 g, all reproduced at the Teaching and Research Farm of the University of Dschang were used for testing.

Experimental design

The animals were randomly divided into 4 groups and maintained in 4 identical boxes. For 60 days, animals in box 1 (control) received orally distilled water (1 ml/kg body weight), while the other three test groups during this same time, received respectively by gavage 80, 100 and 120 µl per kg body weight of guava leaves essential oil. Throughout

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Ingredients	Amount (kg/100kg)
Corn	20,00
Bran wheat	39,50
Palm kernel cake	15,00
Cotton seed meal	8,00
Soybean meal	3,00
Fishmeal	1,00
Bone meal	1,00
Premix 10%	2,00
Salt	0,50
Pennisetum purpurum	10,00
Total	100
Metabolizable energy (Kcal/Kg)	2014,5
Crude protein (%)	17.72
Crude fiber (%)	12.11
Calcium (%)	1.15
Phosphorus (%)	0.87

Table 1: centesimal composition and bromatological characteristics of the ration.

the experimental period, feed (Table 1) and water were provided *ad libitum* to animals.

Serum and organs collections

At the end of treatment, blood was collected by heart puncture from each guinea pig under etheranesthesia. Serum was isolated and stored at -20°C prior to analysis.

After killing the guinea pigs by overdose of ether, organs including testes, epididymis, vas deferens and seminal vesicles were carefully removed, rid of adipose tissue, blotted dry and weighed separately. The left testis was then homogenized in a known volume of cold distilled water, and aliquots of supernatant were kept at –20°C prior to biochemical estimations.

Sperm density and motility

Immediately after the sacrifice, the right epididymis of each animal was minced in 10 ml of warm (36°) NaCl solution for motility evaluation. 20 µl of this mixture were used to count motile and non-motile sperms (X 40). The percentage of motile spermatozoa was determined using the following formula:

Percentage of mobile spermatozoa=[Number of mobile spermatozoa/Total number of counted spermatozoa] X 100

The sperm density was determined in the right cauda epididimis using a Thoma haemocytometer.

Biochemical analysis

Total protein contents of serum and testes were determined using the methods of Biuret [17] and Bradford [18] respectively. Serum and intra-testicular total testosterone were determined using a commercial kit (ELISA AccuDiag™, Diagnostic Automation Inc).

Statistical analysis

Data were submitted to ANOVA test at P<0.05. When differences were significant between means, the latter were separated using the Duncan test. The analyses were performed using SPSS 20.0.

Results

Body and reproductive organs weight

As shown in Table 2, the essential oil had no effect on the body weight of the animals. the relative testicular weight was significantly increased (p<0.05) but the relative epididymal weight, seminal vesicles weight as well as vas deferens weight were not significantly increased with the doses of guava leaves essential oil (p>0.05).

Sperm characteristics

Administration of guava leaves essential oil for 60 days significantly increased the epididymal sperm count dose-dependently compared to the control group. Also, mobility and viability increased as the essential oil dose increased. Except for sperm motility, the values of sperm characteristics parameters were significantly higher (p <0.05) with the highest dose compared to the lowest dose of essential oil (Table 3).

Biochemical parameters

It also comes out from Table 3 that the serum and testicular proteins increased significantly with essential oil dose. When compared to control, the values obtained in groups that received essential oil were significantly higher (p<0.05). Elevated testosterone levels were observed in guinea pigs treated with the essential oil. Between treated groups, testosterone levels were significantly higher with the highest dose (120 µl/kg body weight).

Discussion

The findings of the study indicate that guava leaves essential oil induces an increase in weights of sexual organs such as testis, epididymis, seminal vesicles and vas deferent dose-dependently. These results are comparable to those found by Woode et al. [19] in male rats treated with ethanol extract of the fruits of *Xylopia aethiopica* at doses 30, 100 and 300 mg/kg bw; Ekaluo et al. [15,16] in rats treated with aqueous extracts of *Psidium guajava* leaves (0, 100, 200 and 300 mg/kg bw), and Farouk et al. [20] in male rats treated with essential oil of *Syzygium aromaticum* (0,1 mg/kg bw). The weight, size and secretory function of testes, epididymis and seminal vesicle are closely regulated by androgens [21]. In fact, Androgens, especially testosterone have anabolic properties which are characterized by an increased synthesis of proteins and therefore muscle mass. Androgens then contribute to the increased volume and weight of the testis and epididymis by

parameters	essential oil Doses (µl /kg body weight)			
	0(control) (n=8)	80 (n=8)	100 (n=8)	120 (n=8)
Final body weight (g)	408.51 ± 21.18^{a}	411.03 ± 22.62^{a}	411.76 ± 22.27^{a}	412.60 ± 33.74^{a}
Organ weights (g/100 g bw)				
Testes	0.17 ± 0.01^{a}	0.18 ± 0.05^{a}	0.22 ± 0.4^{ab}	0.25 ± 0.05^{b}
epididymis	0.038 ± 0.004^{a}	0.039 ± 0.018^{a}	0.039 ± 0.011^{a}	0.044 ± 0.008^{a}
Seminal vesicles	0.20 ± 0.1^{a}	0.24 ± 0.1^{a}	0.25 ± 0.2^{a}	0.32 ± 0.2^{a}
vas deferens	0.042 ± 0.008^{a}	0.043 ± 0.010^{a}	0.046 ± 0.024^{a}	0.049 ± 0.010^{a}

(a, b) On the same line, values affected by the same letter were not significantly different (p>0.05); n = number of guinea pigs; Values are presented as Means ± SD.

Table 2: Effect of guava leaves essential oil on the final body weight and the relative weight of the reproductive organs.

paramètres	essential oil doses (µl / kg body weight)			
	0(control) (n=8)	80 (n=8)	100 (n=8)	120 (n=8)
Sperm characteristics				
Sperm density (x 10^6)				
Per cauda epididymis	149.85 ± 5.07^a	151.50 ± 4.52^a	156.16 ± 4.68^b	15877± 6.26^b
per gram of tissue	447.53 ±18.20^a	454.5 ± 20.56^a	471.65 ± 22.57^b	476.30 ±19.71^b
Mobility (%)	89.54 ± 5.15^a	91.68 ± 4.59^{ab}	93.07 ± 3.21^{ab}	94.75 ± 1.93^b
Viability (%)	90.72 ± 3.16^a	91.89 ± 3.59^a	92.01 ± 2.57^a	95.08 ± 1.39^b
Biochemical parameters				
Serum proteins (mg/ml)	11.54 ±3.53^a	16.43 ± 4.62^a	18.14 ± 4.60^b	18.20 ± 5.7^b
Testicular protein (µg/g)	718.7 ± 225.19^a	94511 ± 106.77^b	1157.09 ± 115.82^c	1317.72 ± 71.80^c
Serum testosterone (ng/ml)	2.31± 0.36^a	4.15 ± 0.30^b	4.46 ± 0.29^b	5.08 ± 0.23^c
Testicular testosterone (ng/g)	3.80 ± 0.70^a	4.38 ± 0.46^a	6.05 ± 0.53^b	7.61 ± 0.55^c

(a, b) On the same line, values affected by the same letter were not significantly different (p> 0.05). n = number of guinea pigs; Values are presented as Means ± SD.

Table 3: Effects of guava leaves essential oil on sperm characteristics and biochemical parameters.

stimulating protein synthesis [21,22]. The present study showed a significant increase in serum and testicular protein in guinea pigs which received the essential oil of guava leaves. This increase could be the result of the action of testosterone [23]. The increase in these organs weight could be due to an increase in androgens production. As showed in our study, the essential oil of *Psidium guajava* induced a significant increase in serum and testicular testosterone in treated animals compared to control. This oil may stimulate the synthesis of testosterone by acting on the hypothalamic-pituitary-testicular axis. However, since Uboh et al. [13,14] reported that aqueous extracts of *Psidium guajava* have no effect on LH and FSH production. A direct stimulatory effect of essential oil of *Psidium guajava* on steroidogenesis in leydig cells could be suggested.

This work also revealed a significant increase of epididymal sperm concentration in treated groups. This result could be explained by the elevation of testosterone level. Testosterone is the main male gonadal hormone produced by Leydig cells in the testes in response to LH and under the control of the hypothalamic-pituitary axis [24]. A certain concentration of this hormone is necessary for the initiation and maintenance of spermatogenesis and for stimulating the growth and functioning of the prostate and seminal vesicles [25]. Moreover, it has been reported that the increase in both the sperm concentration and the weight of sexual organs is an indicator of improving male fertility [19]. Sherines et al. [26] showed that the size of the testis is the first evaluation criterion of spermatogenesis because the seminiferous tubules and germinal elements constitute about 98% of the total mass of the testis. Thus, the significant increase in the weight of the testes in guinea pigs that received the essential oil is also related to an increase of sperm production. This observation is similar to those of Akinola et al. [27] and Cajuday and Pocsidio [28] in rats and mice, respectively, treated with aqueous extracts of *Psidium guajava* and *Moringa oleifera*.

The guava leaves essential oil treatment also resulted in a dose-dependent increase in mobility and sperm viability. These results corroborated the findings of Akinola et al. [27] and Uboh et al. [13,14] who reported similar effect of aqueous extract of *Psidium guajava* in rats. This increase can be attributed to the antioxidant properties of the *Psidium guajava* leaves essential oil. In fact, the sperm membrane is rich in polyunsaturated fatty acids, making them especially susceptible to reactive oxygen species (ROS) derived from oxygen metabolism. In addition to an action on lipids, the ROS may also damage DNA and proteins [29]. These molecules can cause lipid peroxidation of the sperm plasma membrane, damage to the structure of the axoneme, problems in the course of capacitation or the acrosome reaction, and loss of motility, which can lead to infertility [30]. However, the essential oil from the leaves of guava contains a number of compounds (phenolic compounds) which confer antioxidant properties, and which would act as scavengers of free radicals [31-33], thus limiting their negative consequences on sperm.

In conclusion, the essential oil from *Psidium guajava* leaves stimulates the production of testosterone, boost sperm production and improve the viability and motility of sperm. Therefore, it can be advised for use by male to improve the reproductive performance.

Competing Interest

The authors declare that they have no competing interest.

Author's Contributions

NF, NS, KA, KP and TJ contributed substantially to conception and design of the study, data analysis and interpretation. NS, TB, NH, NM contributed in data acquisition. NF, NS, TC, KE contributed in drafting the article or revising it critically for important intellectual content. All the authors read and approved the final manuscript.

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