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Oligospermic Effects of Aqueous Extract of *Fragaria ananassa* (Strawberry) On Male Wistar Rats

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ABSTRACT: *Fragaria ananassa* (strawberry fruit) is a popularly consumed fruit that is well known to contain potent antioxidants. The effects of aqueous extract of *Fragaria ananassa* on the sperm parameters and hormones were investigated in male adult Wistar rats. Fifteen (15) male Wistar rats were used for this experiment and were randomly assigned into a control group (A) and two treatment group (B and C) with each group containing five animals (n=5 per group). Animals in group A were given rat feed and water *ad libitum*, those in treatment group B received 200mg/kg body weight of *Fragaria ananassa* (low dose) while animals in treatment group C received 800mg/kg body weight of the extract (high dose). Oral administration of the extract was carried out daily throughout the experimental period which lasted for eight weeks (56 days). At the end of the experimental period, the animals were anesthetised using chloroform and sacrificed. Samples were taken for hormonal assay and sperm analysis. Data were expressed as Mean ± SEM. Significant difference between means were determined by t-test and one-way analysis of variance (ANOVA). Significant difference was expressed as $P<0.05$. Results from sperm analysis showed a dose dependent decrease in the sperm count, motility and normal sperm morphology, and a corresponding increase in abnormal sperm morphology. Hormonal assay revealed a dose dependent significant decrease in testosterone and estrogen level as well as a non-significant increase in the FSH and LH levels. There was also a significant elevation of prolactin and progesterone level. It can be concluded that aqueous extract of *Fragaria ananassa* decreased fertility indices in male rats by affecting sperm parameters and altering pituitary gonadal axis hormones.

Keywords: *Frageria Ananassa*, Oligospermia, Hormones, Sperm Parameters

Introduction

Hormones are indispensable in the control and regulation of fertility. They are produced by endocrine glands and released to target organs on stimulation via negative or positive feedback mechanisms (McLachlan *et al*.; 2002). The gonads in males is one of such target organs and a simultaneous endocrine gland which secrete testosterone in response to the pituitary hormones, FSH and LH (Pierce and Parson, 1981). A variation in the hormonal axis of the hypothalamus, pituitary gland or the gonads may greatly impede on fertility by down-regulating or up-regulating the hormones reaching target organs and their corresponding effects (Holdcraft and Braun, 2004; Pinilla *et al.*, 2012). This has facilitated the need for gonadotropic regulators either as boosters or suppressors of hormone production. In a bid to develop new orally active gonadotrophic agents from plants, a comprehensive screening is being carried out on plants (Yamada, 1998).

Strawberry (*Fragaria ananassa)* is an herbaceous plant that belongs to the genera Fragaria. It is a popular plant whose fruit is widely consumed. Like other common fruits, strawberry is rich in antioxidants like ascorbic acid (vitamin C), folic acid and essential oils (Elkhadragy *et al*, 2017). It is also a rich source of vitamins like thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A, vitamin E and minerals like iodine, magnesium, copper, iron, and phosphorus (Giampieri *et al*., 2012). Strawberry seeds on the other hand in addition to the mineral and vitamin contents are rich sources of essential polyunsaturated fatty acids that cannot be synthesized by the human body (Pieszka *et al*., 2013). Extract from strawberry fruit have been reported to contain a large amount of natural polyphenolic compounds such as flavonoids (mainly anthocyanins with flavonols constituting a fraction), tannins (in the form of ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic and hydroxycinnamic acids) (Proteggente *et al*., 2002).

Studies show that crude extract of berry fruits significantly reduced the oxidative stress in neuronal cells and brain tissues of treated rats (Fuentealba *et al*, 2011). This potent anti-oxidative effect on the nervous tissues was due to the ability of the extract to cross the blood brain barrier as was confirmed by Malin *et al*. (2011). Reports have shown the

cancer-suppressive effect of strawberry on the progression of experimentally induced tumours in animals (Han *et al*., 2005). Reports on the antioxidant effects of methanolic strawberry extract revealed an increase in testosterone level of rats whose testes were oxidatively damaged by cadmium (Mohammed *et al.*, 2017). Considering the strong antioxidant potential of *F. ananassa* and its ability to cross the blood brain barrier. This study was undertaken to unravel the possible influence of its aqueous extract on sperm parameters and hormones of the pituitary gonadal axis.

Materials and methods

Plant material: Strawberry fruits were procured from a fruit shop in Warri, Delta State, Nigeria and was identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. The fruits were chopped into pieces and milled into a solution using 300 ml of distilled water as diluent in an electric blender. The solution was then passed through white filter paper to separate the residue from the filtrate. The filtrate was subsequently concentrated to 80 mg/l at a low temperature, under reduced pressure using freeze dried technique. The extract was preserved in a refrigerator from which fresh solution was prepared using distilled water when required for use.
Animals: The experiment was performed with Fifteen (15) adult male albino rats weighing between 200-250 g obtained from the Animal house of the Department of Anatomy, University of Benin, Benin City and kept at the Animal Care Unit of the Department. The animals were allowed to acclimatize to the laboratory condition (temperature 24-28°C and 12 hours light-dark cycle) for fourteen days before commencement of the experiment with free access to rat chow (Top feeds Nigeria) and water *ad libitum* throughout the study period.
Experimental design: Animals were randomly assigned into a control group (A) and two treatment groups (B and C) with each group containing five animals (n=5 per group). Animals in group A were given rat feed and water *ad libitum*, those in treatment group B received 200 mg/kg body weight of *Fragaria ananassa* (low dose) while animals in treatment group C received 800 mg/kg body weight of the extract (high dose). Oral administration of the extract was carried out daily throughout the experimental period which lasted for eight weeks (56 days). The body weight of the animals were also recorded. At the end of the experimental period, the animals were anesthetised using chloroform and sacrificed.
Sperm motility, count and morphology: After anesthetizing the rats, their testes and epididymis were exposed by abdominal incision, and spermatozoa were expunged out by cutting the distal end of the cauda epididymidal tubule. Spermatozoa within the epididymal fluid was diluted with phosphate buffer. A drop of the solution was put on a Neubauer chamber to assess sperm motility (Amelar *et al*, 1973). Both motile and immotile spermatozoa were counted in different fields to determine the percentage of motile sperms. Sperm morphology were scored in percentages and graded as normal or abnormal cells. Sperm count was determined in the same way, with the exception that 1% formalin solution was used instead of phosphate buffer and the count was expressed as millions of cells/mm^3 (Besley *et al*., 1980).
Hormonal assay: Blood samples were collected by cardiac puncture technique in centrifuge tubes. The blood was allowed to stand for 10 minutes to clot at room temperature and centrifuged at 3500 rev/min for 10 minutes. The serum was then tipped into a separate vial and later subjected by ELISA method for assessment of Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, Testosterone, estrogen (estradiol) and progesterone.
Statistical analysis: Data were expressed as Mean ± SEM. Significant difference between means were determined by t-test and one-way analysis of variance (ANOVA). Significant difference was expressed as $P<0.05$.

Results

There was no significant difference ($P<0.05$) in sperm progressive motility, non-progressive motility and immotility in the treatment groups B and C compared to control group even though there was a dose dependent change (Table 1).
A significant decrease ($P<0.05$) was observed in the total sperm count and percentage of normal sperm morphology in treatment groups B and C compared to control group. The decrease observed was in a dose dependent manner (Table 1).
There was a significant increase ($P<0.05$) in the percentage of abnormal sperm cells in a dose dependent manner this was seen as headless cells in low dose treated group B while high dose treated group C revealed tailless cells as shown in Table 1 below.
Data from hormonal profile, revealed that the level of pituitary-gonadal axis hormones were generally affected in the serum. This was observed as a slight non-significant decrease ($P<0.05$) in testosterone and estrogen level and a slight elevation of FSH, LH, prolactin and progesterone level in the group treated with low dose of *F. ananassa* (group B).
On the other hand, the group treated with high dose of the extract (group C) revealed a significant decrease in the testosterone and estrogen levels and a significant increase in progesterone and prolactin levels with a non-significant increase in FSH and LH levels as shown in Table 2 below.

Table 1: Summary of mean sperm parameters in rats treated with aqueous leaf extract of *F. ananassa.* Values given represent the Mean±SEM

Semen Parameters	Group A (control)	Group B (200mg/kg b.wt)	Group C (800mg/kg b.wt)
Total Sperm Count ($\times 10^6$)	36.00±20.00	25.67±6.69*	13.00±3.00*
Sperm morphology (% Normal)	93.33±3.33	73.33±2.35*	66.67±3.00*
Sperm morphology (% Abnormal)	6.67±4.30	26.67±3.43*	33.33±2.53*
Sperm PM (%)	76.67±6.67	60.00±11.55	56.00±6.00
Sperm NPM (%)	13.33±6.67	16.67±3.33	18.70±3.33
Sperm IM (%)	10.00±0.00	20.33±8.82	23.33±3.33

**(p<0.05) significantly different from the control, PM-progressive motility, NPM-non-progressive motility, IM-immotility*

Table 2: Comparison of mean hormonal values of male rats treated with Low dose (Group B) and High dose (Group C) of *F. ananassa* to Control (Group A)

Hormones	Group A (control)	Group B (200mg/kg b.wt)	Group C (800mg/kg b.wt)
Testosterone (mg/ml)	0.37±0.19	0.29±0.00	0.17±0.10*
Estrogen (ng)	106.33±3.33	98.67±18.81	67.00±6.29*
Progesterone (ng)	4.33±0.67	8.63±0.61	21.93±0.59*
Prolactin (ng)	0.23±0.07	0.40±0.06	0.73±0.03*
FSH (U/L)	0.17±0.03	0.27±0.17	0.37±0.17
LH (U/L)	0.10±0.00	0.20±0.00	0.27±0.62

**(p<0.05) significantly different from the control, FSH - Follicle stimulating hormone, LH - Luteinizing hormone*

Discussion

Results from the present study shows that aqueous leaf extract of *Fragaria ananassa* caused a significant decrease in sperm count, sperm motility and normal sperm morphology in a dose dependent manner. There was also an increase in the sperm abnormal morphology and immotility. Reduction in sperm count may possibly be due to spermatogenic arrest by the extract in the testes or may be due to oxidative damage to germ cells in the seminiferous tubules or epididymis leading to generation of free radical products which exert a detrimental effect on spermatogenesis (Ghosh *et al*., 2002b). The decreased sperm motility and increased abnormality could be attributed to the direct effect of the extract on matured sperm cells stored up in the epididymis which may reduce the fertilizing potential of spermatozoa (Niketan *et al*., 2000). Germinal cell apoptosis is an important mechanism for spermatogenesis (Kierzenbaum, 2001). Current evidence link apoptotic cell death in sperm cells with typical semen parameters. Therefore an alteration of the apoptotic process in spermatozoa will be associated with various forms of abnormal morphology including head and tail defects (Somasagara *et al.,* 2012). This corroborates the decreased sperm parameters and the headless and tailless abnormal spermatozoa observed in this study.

Hormonal assay revealed a significant decrease in the serum level of testosterone and estrogen in a dose dependent manner in rats treated with aqueous leaf extract of *Fragaria ananassa*. Testosterone is required for the growth and development of male reproductive organs. It is also responsible for the maintenance of spermatogenesis (Mooradian *et al.*, 1987). Results from this study showed significant elevation in prolactin and progesterone levels. Hyper-prolactinemia has been associated with decreased testosterone levels by unknown mechanisms (El-Mougy *et al*., 1991). Progesterone is a necessary substrate for the synthesis of testosterone and estrogen (Krester and O' Donnel, 2013). The reduced level of testosterone observed in this study could be attributed to hyper-prolactinamia whereas the decreased level of estrogen observed could imply inhibition of enzymes responsible for the conversion of testosterone into estrogen. The elevated level of progesterone observed may have been due to inhibition of testosterone and estrogen production since progesterone is a necessary substrate for their production (Cheng *et al*., 2010). The hormonal findings, substantiates the results of sperm parameters observed in this study. Although, this result is at variance with a similar study performed by Jiwon *et al.* (2012) where extracts of raspberry which is closely related to strawberry, caused an increase in serum testosterone level. His findings could possibly be due to the varying concentrations of phytochemicals present in the two species.

Results from gonadotropins FSH and LH was seen to be elevated irrespective of the significant decrease in testosterone level suggesting a negative feedback mechanism along the pituitary-gonadal-axis. The rise of FSH by itself is of critical importance in the initiation and expression of spermatogenesis in mammals, whereas, LH is required for the production of testosterone (Sharpe, 1989). From this result, it is clear that the decreased sperm parameters observed in this study were mediated by endocrine regulators as was evidenced by the increase in serum prolactin level.

It can therefore be concluded that aqueous leaf extract of *Fragaria ananassa* induces oligospermia via hormonal mechanisms by inducing hyperprolactinemia and decreasing testosterone levels. It is therefore recommended that further studies be carried out to investigate its mechanism of action.

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