



Effects of Smoothies on Semen Parameters, Nitric Oxide and Total Protein in Male Wistar Rats

¹**Gbaranor K. B.**, ¹Adomale L. F., ¹Tamuno-Opubo A. ²Dimkpa B. M., ¹Emmanuel D. F., ³Baridam G. D., ¹Chris-Biriwo H., ⁴Dimkpa C., ⁵Amuduaghan A. E., ²Oyadotun M., ²Tobin B., ²Anika Ifeoma Eunice., ¹Nelson I. E

¹Department of Human Physiology, College of Medical Sciences, Rivers State University, Port Harcourt, Nigeria

²Department of Community Medicine, University of Port Harcourt Teaching Hospital, Rivers State, South-South, Nigeria

³Department of Obstetrics and Gynaecology, University of Port Harcourt Teaching Hospital, Rivers State, South-South, Nigeria

⁴Department of Paediatrics & Child Health, College of Medical Sciences, Rivers State University, Rivers State, South, Nigeria

⁵Department of Family Medicine, University of Port Harcourt Teaching Hospital, Rivers State, South-South, Nigeria

*Corresponding Author: barinua.gbaranor@ust.edu.ng

Abstract:

Fruits are very important to humans and they have several essential nutrients that are important to improve their overall health. The aim of this study is to investigate the effects of smoothies (banana, apple and pineapple) on semen parameters and nitric oxide in male Wistar rats. Thirty (30) animals weighing 130kg to 180kg were randomly selected into 6 groups with 5 animals per group. Group 1 received 5mls of distilled water, group 2 received 1ml/kg (low dose) of smoothies, group 3 received 2ml/kg (medium dose), group 4 received 3ml/kg (high dose), group 5 received 400mg/kg of monosodium glutamate, group 6 received 400mg/kg of monosodium-glutamate and 3ml/kg (high dose) of smoothies co-administered. Administration was carried out for 14 days and on the 15th day, the animals were sacrificed, semen was harvested and 5ml blood was collected via cardiac puncture. Statistical analysis was done using ANOVA and expressed as Mean±SEM. Statistically $P < 0.05$ was said to be significant. SPSS version 26 was used. Results from the study showed significant increase in sperm morphology and sperm volume and decrease in sperm count and sperm viability. There was significant increase in the level of nitric oxide dose-dependent and thus could aid erection in male.

Keywords:

Effects, Smoothies, Semen, Parameters, Nitric Oxide.



Introduction

The inability to become pregnant after a significant duration of sexual activity without the use of contraception is known as infertility resulting from changes in reproductive factors (Santiago, *et al.*, 2000). According to the report by World Health Organization (WHO) (2003) the annual number of infertile couples globally was found to be 50-80 million. Infertility can have a feminine or masculine origin, with the male factor only present in one third of cases (Babakhanzadeh *et al.*, 2020). Recent meta-analysis studies by researchers show that male's factors are present in 20–70 percent of infertility cases (Agarwal *et al.*, 2015; Qi *et al.*, 2016). A multitude of causes and risk factors contribute to the increasing incidence of male infertility (Salonia *et al.*, 2021) which can be stratified as congenital, acquired, and idiopathic. Environmental or occupational exposure to toxic chemicals (Ma *et al.*, 2019) and various lifestyle factors eg, smoking (Taha *et al.*, 2012; Sharma *et al.*, 2016), alcohol consumption (Ricci *et al.*, 2017) recreational drug use (Gundersen *et al.*, 2015) obesity (Eisenberg *et al.*, 2013) and psychological stress (Nargund, 2015) are all potential risk factors for male infertility (Durairajanayagam, 2018). Most of the healthy nutrients in our smoothies will come from fruit or vegetables used. Benefits of a diet high in fruits and vegetables include lower risk of diseases such as diabetes, obesity, cancer, and cardiovascular disease (Slavin & Lloyd, 2012).

Semen, also known as seminal fluid, is an organic fluid that is emitted from the male reproductive tract and most of the fluid in semen is made up of secretions from male reproductive organs. It contains citric acid, free amino acids, fructose, enzymes, phosphorylcholine, prostaglandin, potassium and zinc (Zinc, 1999). Factors that can lead to abnormally shaped spermatozoa may include; increased testicular temperature, exposure to toxic chemicals, infection and genetic traits. An abnormally shaped sperm is known as teratozoospermia or teratospermia (Ou, M., 2009). NO seems to play a major role in the regulation of sperm motility, hyperactivation, capacitation, and fertilization (Banihani and Aljabali, 2020).

Materials and Methods

Fruit Collection and Identification

Fresh fruits were obtained from mile 3 market in the month of October, 2023. Identification of the fruits was done by Dr. M. G. Ajuru of the department of plant science and biotechnology, Faculty of Science, Rivers state university. The fruits were registered with the code number RSUHPb0155 for banana, RSUHPb0154 for pineapple, and RSUHPb0153 for apple.

Fruit Preparation

Washed banana, pineapple and apple fruits were cut into medium sizes and Poured the fruits into the fruit blender and plugged the blender to an electric source. 250ml of water was added and blended for 5 minutes until it becomes smooth. The blender was Unplugged from electric source and the smoothly blended smoothies was turned into a beaker and used for administration.

Animal and Management

A total of thirty (30) male Wistar rats between 130g to 180g of weight were obtained from animal facility, Faculty of Basic Medical Sciences, Rivers State University, Port Harcourt. The rats were housed under standard laboratory conditions with controlled temperature, humidity. They were provided with standard rat chow and water. The animals were weighed before and after administration of smoothies.

Study Design

The rats were randomly selected into six(6) experimental groups with five(5) rats per group and administration of substances for 14 days.

Group 1: was administered 5ml of distilled water for 14 days

Group 2: was administered 1ml of smoothies (low dose) for 14 days

Group 3: was administered 2ml of smoothies (medium dose) for 14 days

Group 4: was administered 3ml of smoothies (high dose) for 14 days

Group 5: was administered 400mg of monosodium glutamate for 14 days

Group 6: was administered 400mg of monosodium glutamate + 3ml of smoothies (high dose) for 14 days

Sample Collection

Administration of smoothies (banana, apple and pineapple) was done for 14 days and on the 15th day, the animals were anaesthetized with chloroform soaked in cotton wool and thereafter, sacrificed.

Semen Analysis

Heemocytometer Method of Semen Analysis

1. Lacerated the epididymis to press out the semen.
2. Emulsified with 0.5% Eosin
3. Examined using x10 & x 40 objective lens
4. Examined 10-12 fields to check for viable cell which is the % of stain cells as against the unstain ones.
5. Examined 10 -12 field to check for normal cells as against the abnormal ones in %. Did the same for active motile cells, sluggish cells and the death cells.
6. The sperm count was done using counting chamber, 1:20 dilution of the semen was done using formal saline.
7. Assembled the chamber and filled the chamber with the diluents.
8. Counted the 4 x 16 squares and multiplied by 100,000.
9. Example: no of cells counted x 100,000 = sperm count.

Statistical Analysis

Statistical analysis was done using ANOVA and expressed as Mean±SEM. The statistically p less than 0.05 was said to be significant. SPSS version 26 was used

Ethical Approval

Application for ethical approval to the Research Ethics Committee, Faculty of Basic Medical Sciences, Rivers State University was approved with the number RSU/FBMS/REC/23/026.

Results

The result obtained for the viability, showed that the group that was administered with 1ml, 2ml, 3ml and 400mg of MSG significantly decreased compared to control. The result obtained for volume showed that the group that was administered with 1ml, 3ml and co-administration of MSG + 3ml significantly increased. While the 400mg of MSG group significantly decreased compared to control.

The results obtained for sperm count showed that the group that was administered with 1ml, 400mg of MSG, and co-administration of MSG + 3ml significantly decreased compared to control (Figure 1).

The result obtained for the normal morphology, showed that the group that was administered with 3ml of smoothies significantly increased compared to control. The result obtained for the active cells showed no significant difference. The result obtained for the dead cells showed that the group that was administered with 3ml of smoothies significantly decreased compared to control group (Figure 2).

The result obtain for total protein, showed that the group that was administered with 1ml, 2ml, 3ml, 400mg/ml/kg of MSG and the co administered with MSG significantly decreased compared to control. The result obtained for nitric oxide showed that the group administered with 1ml, 2ml, 400mg/ml/kg of MSG and co-administration of MSG + 3ml significantly decreased. While the group administered with 3ml of smoothies significantly increased compared to control (Figure 3).

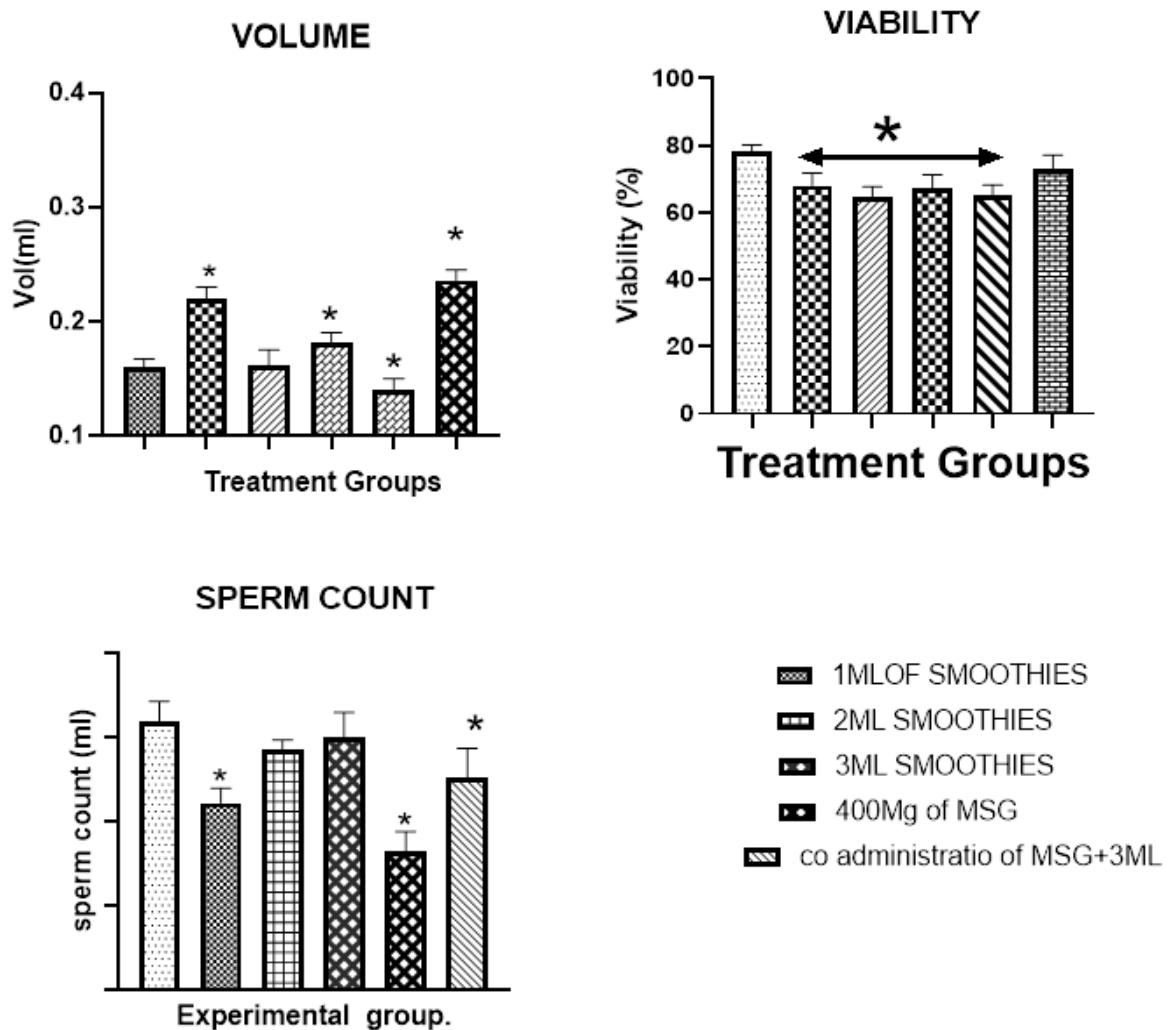


Fig 1: Effects of different doses of smoothies on the volume, viability and sperm count of male Wistar rats

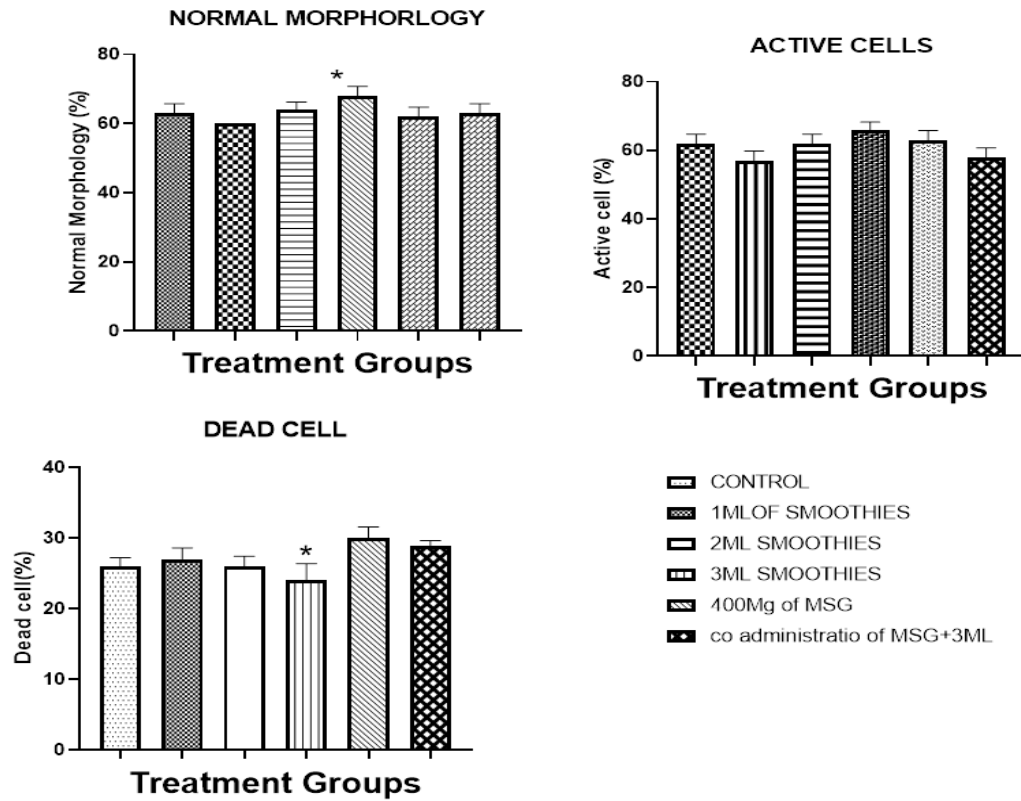


Fig. 2: Effects of different doses of smoothies on the normal morphology, active cells and dead cells of male Wistar rats

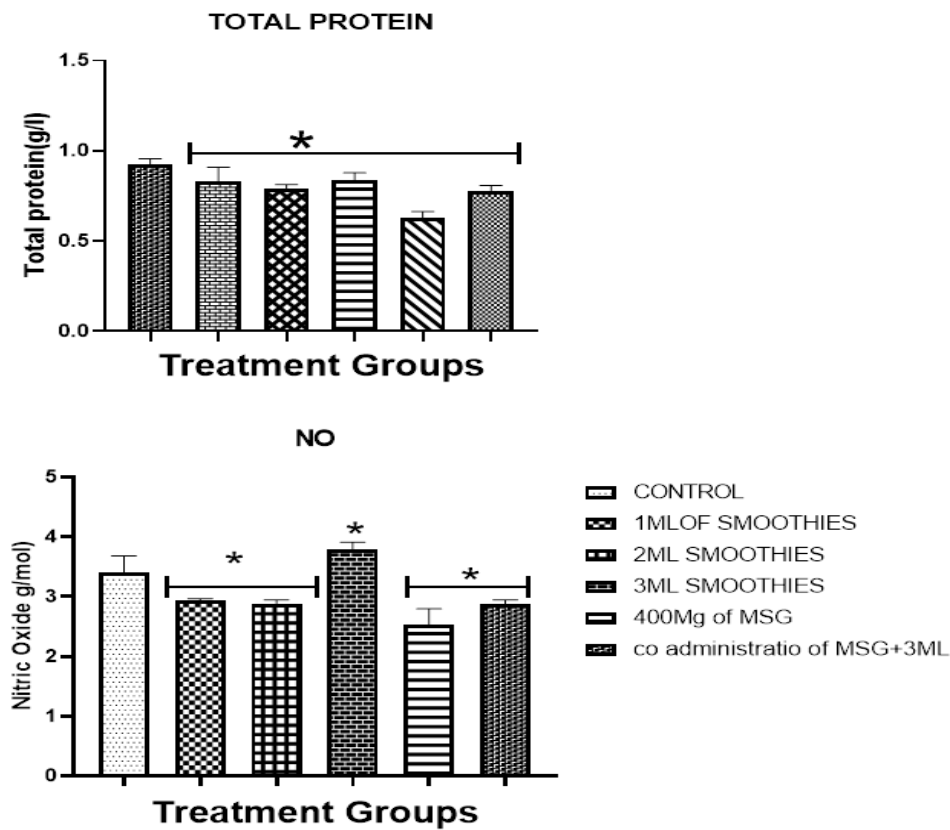


Fig. 3: Effects of different doses of smoothies on total protein and nitric oxide of male Wistar rats

Discussion

Fruits are natural food that contained phytonutrients which provide the role play by the fruits in the body. Fruits are widely eaten across the globe and they provide the body with minerals and vitamins. These fruits are eaten singly or combined. Some of the fruits are prepared as juice or smoothies and some are eaten ripe or some unripe. Smoothies are naturally prepared juice that comes in varieties. The cardinal aspect of smoothies is the presence of ripe banana that make it thick when prepared and this differentiate it (smoothies) from other natural fruit's juice. Most African men depends on fruits intake to improve the quality of their semen. However, these men do not know whether these fruits could be harmful or not. Smoothies are widely consumed by men mostly to improve sperm volume and no documentation and the reason for this study.

The study revealed that the volume of sperm significantly increased in the groups of rats given 1ml/kg and 3ml/kg of smoothies. This increase in the volume of sperm indicates that smoothies (Banana, pineapple and apple) contain phytonutrients that increase the volume of sperm. The volume of sperm reduces significantly in the group administered with 400mg of monosodium glutamate. This reduction in the volume of sperm could be due to substance found in MSG. This mean that the substance MSG may have damage the sperm cells resulting in the reduction of the volume. This study agreed with previous studies that revealed that, MSGs are also implicated or suspected in male sterility by triggering testicular hemorrhage, deterioration and change of sperm cell number and morphology (Nayanatara *et al*, 2008; Igwebuike 2011) and that MSGs has dangerous impact on testis by triggering notable oligozoospermia and increases non-standard sperm morphology in amount-dependent male rats (Onakewhor *et al*, 1998). However, when MSG 400mg/kg and 3mls of smoothies was co-administered, the volume of sperm increases significantly. This shows that the phytonutrients in the smoothies reduces the effect of MSG on sperm volume.

The results also shows that the viability of sperm significantly reduces in the groups treated with 1ml/kg, 2ml/kg, 3ml/kg and MSG 400mg/kg when compared with the control. This indicate that smoothies may affect the viability of sperm. Also, sperm count decreases significantly in the group treated with 1ml/kg of smoothies when compared with the control. This decrease in sperm count could be due to dose dependent. There is further reduction in sperm in the group treated 400mg/kg of MSG and this reduction could be due to MSG which previous studies have implicated in the damage of sperm cells. However, when smoothies and MSG was co-administered, the sperm count increase to certain level above the groups treated with 1ml of smoothies and MSG 400mg/kg. This indicate that the smoothies and the MSG may have synergistic effect. In the group treated with 3mls of smoothies, there was significantly increase in the normal morphology of the sperm when compared with the control group. This increase in normal morphology could be due to dose dependent. The group treated with MSG 400mg/kg has its dead cells significantly decrease when compared with control. This shows that MSG contain substance that may cause dead cells.

The role of nitric oxide (NO) in erectile physiology is well known and NO activates relaxation of corporal cavernosal smooth muscle tissue resulting in increased blood flow into the penis resulting in an erection (Kelvin P Davies, 2015). Erection is aid by nitric oxide (NO) and its binds with the heme iron guanylate cyclase and activates the enzyme, guanylate cyclase. Guanylate cyclase converts GTP into cGMP which closes C^{2+} channels. Impaired NO bioactivity is a major pathogenic mechanism of erectile dysfunction (Burnett, 2007). The study revealed that, the nitric oxide levels in the groups treated with 1ml/kg and 2ml/kg of smoothies is significantly decrease when compared with control and this may affect erection. Again, in the group treated with 3ml/kg of smoothies has its nitric oxide significantly increase when compared with control and this increase could be dose dependent and could also be beneficial to men who could not have penile erection. In the group treated with MSG 400mg/kg and the group treated with MSG 400mg/kg and 3ml/kg of smoothies co-administered also has its nitric oxide levels low when compared with control and this reduction could be due to the presence of MSG.

Total protein (albumin and globulin) are produced by the liver and in the case of a liver damage, production of these proteins are reduced or completely ceased (Sunday, *et al.*, 2021). The concentrations of the total protein, bilirubin and albumin may indicate the state of the liver and the type of damage (Yakubu *et al.*, 2005). The study revealed that all the treated groups has its total proteins level significantly decreased when compared with the control. This decreased in the serum level of total protein indicate that the liver is not protected. The liver is the source of protein production. This shows that the decreased in serum levels of total protein in all the treated groups could be due to the presence of essentials bioactive compounds in the smoothies and this may help to restore and protect the liver.

Conclusion

Smoothies (Banana, pineapple and apple) are widely used across Nigeria to encourage healthy living. The study revealed that smoothies improves sperm morphology and sperm volume and decreases sperm count and sperm viability. Also, the serum levels of total proteins decrease and this may not protect the liver.

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