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Sulaimon Mutiu O.
Department of Statistics & Mathematics Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

Adewunmi Olusola A.
Department of Statistics & Mathematics Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

Mabosanyinje Adedeji
Department of Statistics & Mathematics Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

Adewunmi Adebisi R.
Department of Science Laboratory Technology Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

Effects of Paracetamol (Acetaminophen) Usage on Sperm Count

Sulaimon Mutiu O., Adewunmi Olusola A., Mabosanyinje Adedeji, Adewunmi Adebisi R.

Abstract
Several published studies have connected paracetamol (also named Acetaminophen, a commonly used analgesic and antipyretic medication) with semen quality and male fertility. This research work statistically investigates the effects of paracetamol usage on sperm count by modelling the impact of varieties of paracetamol combinations, different dosage of varieties of paracetamol combinations and the interactions on sperm count. Data for this research was collected primarily via experimental method. A sample of 30 adult male albino rats weighing 160g – 180g was used in lieu of human beings to determine the effect of paracetamol on sperm count. Sperm extraction were carried out after the completion of the expected drug administration period to determine the available sperm count for each rat which were thereafter analysed statistically to make appropriate inferences. One of the results from the findings shows that rats administered 500mg of Orphensic with basal feed approximately produce the least sperm count of 7.500 million on the average. The varieties of paracetamol combinations significantly have different effects on sperm count. Of the ten interactions, significant interaction effects on sperm count is found in 500mg of Standard paracetamol, 500mg of Cenpain, 1000mg of Standard paracetamol and 1000mg of Cenpain. More also, long term paracetamol usage does not reduce body weight. We advise that men especially should be careful in acetaminophen usage without the prescription of their physician. They should be conscious of the level of consumption of parcetamol as it does significantly affect sperm count in general.

Keywords: Count, Effect, Paracetamol, Sperm, Usage

I. Introduction

Infertility is a major problem in up to 15% of the sexually active population and male factor is responsible in 50% of these cases. Paracetamol is a frequently used analgesic and anti-pyretic drug which is widely available without a prescription. According to Medical professionals, it was concluded that long-term use, or large doses of paracetamol can cause harm to the body, although many mistakenly believe it to be completely harmless. Paracetamol is the main ingredient in everyday medications such as cold and flu remedies. Although discovered in the 1890s and marketed as a painkiller since the 1950s, exactly how it relieves pain was unknown (Abarikwu S. O., 2013).

This study, funded by the Medical Research Council (MRC) and recently published in *Nature Communications*, shows for the first time the principal mechanism of action for one of the most-used drugs in the world. A research team at King's led by *Professor Stuart Bevan*, with colleagues from Lund University in Sweden, have identified that a protein called TRPA1, found on the surface of nerve cells, is a key molecule needed for paracetamol to be an effective painkiller. However, as discovered by Suneil Agrawal and Babak Khazaeni (2018), it is estimated that paracetamol poisoning results in 56,000 injuries, 25,000 hospitalizations, and 450 deaths every year, It is also shown that overdosing with acetaminophen can cause hepatic necrosis in both humans and laboratory animals and prolonged human use has been implicated in chronic renal disease necrotic changes in lung, testis, lymphoid tissue of mice and asthma in children.

High doses of acetaminophen have also been reported to lead to testicular atrophy and decrease of testosterone hormone in human. A previous study was performed to assess the

Correspondence:
Sulaimon Mutiu O.
Department of Statistics & Mathematics Moshood Abiola Polytechnic, Abeokuta, Ogun State, Nigeria

effect of paracetamol by the *Health Sciences students of University of Peradeniya* (Samarawickrama *et al*, 2014) and it was discovered that over-dosage of paracetamol causes liver damage and less renal tubular necrosis. It is the first step on the *WHO (World health organization*) pain ladder and is currently recommended as first-line pharmacological therapy by a variety of international guidelines for a multitude of acute and chronic painful conditions.

The mechanism of paracetamol's analgesic action remains largely unknown, but recent studies demonstrate that paracetamol inhibits prostaglandin production within the central nervous system and within peripheral tissues. Administration of activated charcoal is a useful treatment for paracetamol poisoning. Paracetamol has been around for over 50 years. It's safe and many guidelines recommend it as the go-to treatment. At least, that's the conventional view of the drug. It's a view so ingrained that it's rarely questioned. The trouble is that the conventional view is probably wrong.

In a research paper on paracetamol toxicity published by Ibrahim T. *et al* (2013), it was stated that the use of paracetamol is one of the most common causes of poisoning worldwide. Its poisoning can be due to ingestion of excessive repeated or too- frequent doses. Repeated supra therapeutic ingestion is a significant clinical problem. However, overdose or long term uses of paracetamol have well-known adverse effects including hepatotoxicity, depletion of reproductive competence, alteration of testicular structure and ultra-structure and seminal quality impairment. Acute paracetamol (N-acetylp-aminophenol; APAP) overdose may induce testicular toxicity in humans and experimental animals (Kennon-Mcgill S. and Mcgill M. R., 2018).

According to Amy Dixon J. (2009), the initial step of its toxicity is formation of the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI) by cytochorom P450 which at therapeutic doses is removed by conjugation with glutathione (GSH). High doses of paracetamol result in the depletion of cellular GSH which allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to toxicity. Paracetamol-induced toxicity could also be due to hepatic-derived paracetamol metabolites; particularly GSH conjugates.

Medical professionals have concluded that long-term use, or large doses of paracetamol can cause toxicity, leading to liver failure or even death. It is to these effects that this research work aim to determine the effects of paracetamol usage on sperm count.

II. Statement of the problem

It is shown that overdosing with paracetamol (acetaminophen) can cause hepatic necrosis in both humans and laboratory animals and prolonged human use has been implicated in chronic renal disease necrotic changes in lung, testis, lymphoid tissue of mice and asthma in children. High doses of acetaminophen have also been reported to lead to testicular atrophy and decrease of testosterone hormone in rat and human. The problem of this research work is determining statistically, the effect of paracetamol usage on sperm count.

III. Aim and objectives of the study

The purpose of this study is to statistically determine the effects of paracetamol usage on sperm count by modelling the effect of varieties of paracetamol combinations, different dosage of varieties of paracetamol combinations and the interactions on sperm count.

The objectives are:

1. To determine if there is significant difference in the effect of varieties of paracetamol combinations on sperm count.
2. To determine if there is significant difference in the effect of different dosage of varieties of paracetamol combinations on sperm count.
3. To determine if there is significant interaction effect between varieties of paracetamol combinations and different dosage.
4. To determine if long term paracetamol usage reduces body weight.

IV. Research questions

The following research questions shall guide the study and sharpen the course of the investigation:

1. Is there a significant difference in the effect of varieties of paracetamol combinations on sperm count?
2. Is there a significant difference in the effect of different dosage of varieties of paracetamol combinations on sperm count?
3. Is there a significant interaction effect between varieties of paracetamol combinations and different dosage of varieties of paracetamol combinations?
4. Does long term paracetamol usage reduce body weight?

V. Research hypotheses

Based on the conceptual frame work and objectives of this research work, the following hypotheses direct the conduct and analysis of this research.

The research hypotheses are:

1. H_{01}: $A_i = 0$ (There is no significant difference in the effect of varieties of paracetamol combinations on sperm count)
2. $H_{02:}$ $B_j = 0$ (There is no significant difference in the effect of different dosage of varieties of paracetamol combinations on sperm count)
3. H_{03}: $(AB)_{ij} = 0$ (There is no significant interaction effect between A and B)
4. H_{04}: $\mu_d \geq 0$ (Long term paracetamol usage reduces body weight)

where

$A_i -$ Effect of varieties of paracetamol combinations. For $i = 1, 2, \ldots, 5$

$B_j -$ Effect of different dosage of varieties of paracetamol combinations. For $j = 1, 2$

$(AB)_{ij} -$ Interaction effect between A and B.

VI. Scope of the study

30 adult male albino rats weighing 160g – 180g were used in lieu of human beings to determine the effects of paracetamol usage on sperm count. Being a clinical trial experiment, use of rats as case study as against humans is of importance to prevent unforeseen and irreversible side effects in the human system. In addition, human beings and rats are more alike than different because we have the same basic physiology similar organs and similar body plans. We both control our body chemistry using similar hormones, both nervous system work in the same way and reaction to

injury and infection is similar.
The rats were randomly divided into 5 groups and were fed with basal feed for 1 week. Four of the five groups received different dosage of varieties of paracetamol combinations, while the fifth group (control group) were only fed with basal feed.
Sperm extraction were thereafter carried out after the completion of the expected drug administration period to determine the available sperm count for each rat which were thereafter analysed statistically to make appropriate inferences.

VII. Literature review

According to a research carried out by (*Aksu et al*, 1992) they claimed that their study demonstrates decrease in sperm motility and live sperm rate in male rat by consumption of paracetamol. Their findings showed that almost all of the sperm parameters were decreased following consumption of normal and high dosage of paracetamol in three period of spermatogenesis in mice. A similar study has shown that the treatment of paracetamol causes a significant decrease in sperm motility and sperm count in rat, it is also shown that paracetamol may cause a significant decrease in morphologically normal spermatozoa. These effects could be due to impacts of paracetamol on testis and epididymis.
Ratnasooriya and Jayakody (2001) also declared that long-term administration of high doses of paracetamol damages the reproductive competence of male rats. They presented that these effects are reversible and was not due to a general toxicity but due to an increase in oligozoospermia, deficiencies of normal and hyper-activated sperm motility, and reduction in the fertilizing potential of spermatozoa. This is also an investigation on the relationship between sperm chromatin condensation and short and long-term paracetamol administration in normal and high doses. Regarding to the DNA integrity tests, firstly in SCD test, they found a significant difference among groups in three period of spermatogenesis. This showed that paracetamol consumption in normal and high doses may cause sperm DNA fragmentation.
Oligospermia is a male fertility issue defined as a low sperm concentration in the ejaculate.
Low sperm concentration or low sperm count means that the fluid (semen) ejaculated during an orgasm contains fewer sperm than normal. As defined by the World Health Organization (WHO) 1999, a low sperm count is less than 20 million sperm/mL.
In David W. Russell's (2017) book, "Effects of Paracetamol on Adults", he revealed that in a study, published in the journal proceedings of the National Academy of Sciences, Danish and French scientists carried out a series of trials to see how paracetamol affects adults. In tests on 31 young men, two-week use of the drug affected levels of crucial male hormones, including testosterone and lab experiments also show testicular tissue exposed to the painkiller made less testosterone and damaged its ability to make sperm.
Researcher David Møbjerg Kristensen (2018), from the University of Copenhagen, said: "Our lab tests show that when you hit the testes with a compound like paracetamol, it results in a reduction in all the main male hormones.
In CMA3 staining, in the first 35 days they found significant differences between groups only when high dosage of paracetamol was treated. On the other hand, there were significantly differences between groups in the rates of sperm protamine deficiency in both normal and high dosage of paracetamol after 70 and 105 days. Thus, it seems that long-term use of paracetamol has detrimental effects on histone–protamines replacement during the testicular phase of sperm chromatin packaging and cause sperm protamine deficiency in mice.
There are some possible reasons for sperm DNA damage following acetaminophen treatments. It is shown that acetaminophen causes an inhibition of both DNA replication and DNA repair by a specific inhibition of enzyme ribonucleotide reductase. In fact, these effects provide a reason for acetaminophen's ability to make sister chromatid exchanges, micronuclei, chromosomal amber.

Levels of low sperm count

Definition	Sperm Concentration in Ejaculate
Mild Oligospermia	10 million to 20 million sperm/Ml
Moderate Oligospermia	5 million to 10 million sperm/Ml
Severe Oligospermia	100000 to 5 million sperm/Ml
Cryptoozospermia	Below 100000-rare sperm
Azoospermia	0 sperm

OLIGOSPERMIA means low volume of sperm.

The table above shows how low sperm counts are described: In the statement of world health organization (WHO) on semen quality (2010), the WHO now considers a sperm count of 15 million sperm/mL to be low for fertile men.
From Dr. Turek's web blog, it was shown that low sperm counts can also mean that a patient is at higher risk of developing both testicular cancer (2.8x higher) and prostate cancer (2.6x higher) later in life. In this sense, then, a low sperm count can be a natural biomarker of future health in men. For these reasons, all infertile men with a low sperm count should be evaluated with a thorough history and physical examination by a specialist.
It has been reported that high dose of paracetamol (>2000mg per day) does increase the risk of gastrointestinal complications such as stomach bleeding (Rodriguez and Hernadez-Diaz, 2001).
It is also effective in the treatment of musculoskeletal pain in dogs (Oyedeji *et al*, 2013). There is inadequate evidence in experimental animals for the carcinogenicity of acetaminophen. In rats fasted 24 hours and given a single dose of acetaminophen (2 g/kg) by gavage, liver necrosis around the central vein was noted at 9-12 hours and was much more extensive at 24 hours after treatment.
In mice after dietary exposure to acetaminophen up to 6400 mg/kg daily for 13 weeks hepatotoxicity, organ weight changes and deaths were observed.
Cats are particularly susceptible to acetaminophen intoxication, developing more diffuse liver changes, while hepatic centrilobular lesions found in dogs.
High doses of acetaminophen caused testicular atrophy and delay in spermatogenesis in mice. Furthermore, reductions in the fertility and neonatal survival in mice were seen in the F0 generation and decreases in F1 pup weights were found at acetaminophen dose 1430 mg/kg.
What is clear and has been confirmed by Dr. Turek's research is that a low sperm count can be an indicator of a general medical problem or a genetic condition. In 2% of men, low sperm counts may be due to hormonal imbalance

from prolactinoma. In addition, one of the most common causes of low sperm counts is a varicocele, a surgically treatable condition.

Increasingly, genetic abnormalities are being found in men with severe oligospermia. Missing regions on the Y chromosome (microdeletions) occur in 6% of men with low sperm counts and 15% of men with no sperm counts.

In addition, 2% of men with low counts and 15-20% of men with no sperm counts will harbor chromosomal abnormalities detected by cytogenetic analysis (karyotype). Freezing of spermatozoa from infertile men may affect sperm motility, morphology, DNA integrity, mitochondrial activity, and viability. Different studies have demonstrated that cryopreservation of spermatozoa induces reactive oxygen species (ROS) production.

Semen is the mixture of fluids from the testis and other glands in the male reproductive tract. In fact, the sperm and the fluid from the testes make up only about two per cent of the volume of the semen that is ejaculated. Sperm move up the epididymis in this small amount of fluid and then mix with larger amounts of fluid from the seminal vesicles (60% of the semen), the prostate (30% of the semen) and other smaller glands (8% of the semen), before ejaculation.

Sperm production

Spermatogenesis (sperm production) is a continuous process with millions of sperm being made each day after puberty. Within the testis, sperm can be at different stages of development. It takes about 70 days to complete the development of sperm that are able to swim and fertilise an egg.

A man's reproductive system is specifically designed to produce, store, and transport sperm. Unlike the female genitalia, the male reproductive organs are on both the interior and the exterior of the pelvic cavity. They include:

- the testes (testicles)
- the duct system: epididymis and vas deferens (sperm duct)
- the accessory glands: seminal vesicles and prostate gland
- the penis

Sperm production occurs in the testicles. Upon reaching puberty, a man will produce millions of sperm cells every day, each measuring about 0.002 inches (0.05 millimeters) long.

There is a system of tiny tubes in the testicles. These tubes, called the seminiferous tubules, house the germ cells that hormones including testosterone, the male sex hormone cause to turn into sperm. The germ cells divide and change until they resemble tadpoles with a head and short tail. The tails push the sperm into a tube behind the testes called the epididymis. For about five weeks, the sperm travel through the epididymis, completing their development. Once out of the epididymis, the sperm move to the vas deferens.

When a man is stimulated for sexual activity, the sperm are mixed with seminal fluid (a whitish liquid) produced by the seminal vesicles and the prostate gland to form semen. As a result of the stimulation, the semen, which contains up to 500 million sperm, is pushed out of the penis (ejaculated) through the urethra.

The process of going from a germ cell to a mature sperm cell capable of egg fertilization takes around 2.5 months. Sperm are produced in the testicles and develop to maturity while traveling from the seminiferous tubules through the epididymis into the vas deferens.

The process of making sperm can be interrupted at various stages for a number of reasons:

- Absence of germ cells (called Sertoli cell-only syndrome): the testis may completely lack the germ cells that normally divide to become sperm. This is a severe problem. If every tubule shows this pattern, the man will be sterile as there are no sperm in the semen or in the testes.
- Maturation or germ cell arrest: sometimes germ cells stop developing and do not become mature sperm.
- Hypospermatogenesis: when the number of sperm made in the testes is lower than normal, smaller numbers, or sometimes no sperm, make it through into the ejaculated fluid.

It is estimated that one in 20 men has some kind of fertility problem with low numbers of sperm in his ejaculate. However, only about one in every 100 men has no sperm in his ejaculate.

The most common cause of male infertility is a problem with making sperm in the testes. Either low numbers of sperm are made and/or the sperm that are made do not work properly.

About two-thirds of infertile men have a sperm production problem. Unfortunately, medical scientists do not yet understand all the details of healthy sperm production. Therefore, the cause cannot be found for many men with a sperm production problem.

Sperm transport problems

Blockages (often referred to as obstructions) in the tubes leading sperm away from the testes to the penis can cause a complete lack of sperm in the ejaculated semen. This is the second most common cause of male infertility and affects about one in five infertile men, including men who have had a vasectomy but now wish to have more children.

Some blockages may be related to congenital problems (that is, being born with the problem) which can be found with specialised tests. Most of the time, men who have a sperm production or transport problem show no obvious signs or symptoms.

Sexual problems: Problems with erections (erectile dysfunction) or ejaculation can affect whether semen is able to enter the woman's vagina for fertilisation to take place. About one in 100 infertile couples has trouble getting pregnant because of erection, ejaculation or other sexual problems.

Paracetamol and male fertility

Couples in which the male partner had high levels of paracetamol in his urine took longer to achieve pregnancy than couples in which the male had lower levels of the compound, according to a preliminary study by researchers at the National Institutes of Health (2006). Paracetamol, also known as acetaminophen, is a non-prescription drug widely used as a pain reliever and fever reducer. It also is one of the compounds produced when the body breaks down aniline, a chemical used to make rubber, pesticides, and colouring agents used in food, cosmetics and clothing. At the same year, the study was published online in the journal of human reproductive Sciences, National Institutes of Health. It was explained that the high levels of paracetamol in the urine of certain men in the study were unlikely to result only from taking medications and were more consistent with those seen from environmental exposure, either to aniline or paracetamol or a combination of the two. The findings could have implications for the amount of paracetamol exposure that is considered acceptable.

The current finding results from the latest analysis of data from the Longitudinal Investigation of Fertility and the Environment (LIFE) study, established to examine how lifestyle and exposure to environmental chemicals may affect fertility. The LIFE study encompasses fertility data from 501 couples enrolled in four counties in Michigan and 12 counties in Texas from 2005 to 2009.

The women taking part in the study ranged from 18 to 44 years of age, and the men were over 18. Each participant provided a single urine sample upon joining the study, which was analyzed to measure its paracetamol concentration.

Women had a higher average level of paracetamol (26.6 mg/mL) than the men (13.2 mg/mL). A high level of paracetamol for the female partner was not associated with reduced chances for pregnancy. However, couples in which the males had high levels of paracetamol (more than 73.5 mg/ml) were 35 percent less likely to achieve a pregnancy, compared to couples in which the males had low levels (less than 5.4 mg/ml.).

The authors stressed that their findings need to be confirmed by larger studies that can better identify the sources of paracetamol, the duration of time the participants are exposed, and the amount of the compound to which they are exposed.

Two-factor factorial experiment

Two-factor factorial experiment involves the simultaneous application of two treatment factors, each at more than one level application. Such experiment requires an analysis that is able to separate the combined treatment effect, its components and thereby allow the significance of effects of each of the two treatment factors, e.g soil and fertilizer, and their interaction to be determined independently (Clive R. Ireland, 2010). Thus the total variation in the data set, described by total sum of squares, is partitioned between each of the two treatment factors and the random or residual variation. In addition, as long as there is interaction present within samples, the interaction between treatments may be analysed as a further source of variation.

In two-factor factorial experiment (with replication), there are, therefore, three separate null hypotheses to be tested:

- H_{01} – there is no effect of treatment A
- H_{02} – there is no effect of treatment B
- H_{03} – there is no effect of treatment A*B

The Concept of Treatment Interaction

When two or more treatment factors are applied simultaneously in a multiple factorial experiment, the possibility of detecting interaction between the treatment factors is evoked. Interaction is the effect that one treatment may have in modifying the response of the subjects to another simultaneous treatment. Sometimes, of course, a negative interaction might occur in which the response to a combined treatment is decreased compared with the sum of the effects of the individual treatments. Some of the different types of interaction that may occur in two-way experiment are:

(a) No effect of factor A
Significant effect of factor B
No significant interaction

(b) Significant effect of factor A
Significant effect of factor B
No significant interaction

(c) No effect of factor A
No effect of factor B
Significant interaction

(d) Significant effect of factor A
Significant effect of factor B
Significant interaction

(e) No effect of factor A
Significant of factor B
Significant interaction

(f) Significant effect of factor A
No effect of factor B
Significant interaction

Interaction between treatments is very important in experimental biology very often the effects of individual treatments will be well known beforehand and it is the question of whether the treatment factors interact in producing a response that then becomes the primary concern of the investigator and the main reason for performing a multiple- factorial experiment.

Assumptions and limitations of Two-way ANOVA

1. The samples are independent.
2. The samples are drawn from a normally distributed population.
3. The samples are drawn from a population that have equal variances.

In two-way factorial experiments, a distinction needs to be made between cases when there is replication and when there is no replication within samples. Clearly a one-way factorial experiment without replication is untenable since the sample means would be based on single observations and no within sample variance would exist.

It is possible, however, to conduct a two-way factorial experiment without replication so that the single values for the different levels of one treatment factor are employed as the replicate for the other treatment factor. However when no replication is present, interaction is not then detectable and the sources of variation are simply the two treatment factors and the residual variation.

The reason for this is fairly obvious, without replication, no within-groups variance can be calculated and therefore the response to the treatment cannot be compared under different application levels of a second treatment. Thus, treatment interaction only becomes a detectable source of variation when sample size is ≥ 3

Replication

It is the repetition of the experimental situation by replicating the experimental unit. In the replication principle, any treatment is repeated a number of times to obtain a valid and more reliable estimate than which is possible with one observation only. Replication provides an efficient way of increasing the precision of an experiment. The precision increases with the increase in the number of observations. Replication provides more observations when the same treatment is used, so it increases precision.

Interaction

The two-way ANOVA subsequently produce three separate values of test statistic F, i.e., F_{ratio} for each of the two treatment factors and F_{ratio} for their interaction, which are used to independently test the three null hypotheses.

Two-factor factorial experiment with 'n' replicates per cell run as a completely randomized design.

A general model will be

$$y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk} \quad \text{\_\_} \qquad (1)$$

$$i = 1, 2, 3, \dots, a$$
$$j = 1, 2, 3, \dots, b$$
$$k = 1, 2, 3, \dots, n$$

where

A_i = The effect of i^{th} level of factor A

B_j = The effect of j^{th} level of factor B

$(AB)_{ij}$ = The effect of interaction between A and B

e_{ijk} = Random error component; where $e_{ijk} \sim N(0, \sigma^2)$

Partitioning the sum of squares

From the equation __(1)

Let $\mu = \bar{y}_{...}$

$A_i = \bar{y}_{i..} - \bar{y}_{...}$

$B_j = \bar{y}_{.j.} - \bar{y}_{...}$

$(AB)_{ij} = \bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}$

$e_{ijk} = y_{ijk} - \bar{y}_{ij.}$

Thus, substituting the notations into the model we have:

$$y_{ijk} = \bar{y}_{...} + (\bar{y}_{i..} - \bar{y}_{...}) + (\bar{y}_{.j.} - \bar{y}_{...}) + (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}) + (y_{ijk} - \bar{y}_{ij.}) \quad \text{\_\_} \qquad (2)$$

$$(y_{ijk} - \bar{y}_{...}) = (\bar{y}_{i..} - \bar{y}_{...}) + (\bar{y}_{.j.} - \bar{y}_{...}) + (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}) + (y_{ijk} - \bar{y}_{ij.}) \quad \text{\_\_} \qquad (3)$$

Let $(y_{ijk} - \bar{y}_{...}) = p$, $(\bar{y}_{i..} - \bar{y}_{...}) = q$, $(\bar{y}_{.j.} - \bar{y}_{...}) = r$,

$(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...}) = s$, $(y_{ijk} - \bar{y}_{ij.}) = t$

So that $p = q + r + s + t$

Squaring both sides, we have:

$$p^2 = (q + r + s + t)^2 \quad \text{\_\_} \qquad (4)$$

That is, $p^2 = q^2 + 2qr + 2qs + 2qt + r^2 + 2rs + 2rt + s^2 + 2st + t^2$ __ (5)

Summing equation ___(5) across i^{th} level of factor A, j^{th} the level of factor B, and n replicates per cell respectively, we have it reduced to:

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} p^2 = bn\sum_{i=1}^{a} q^2 + an\sum_{j=1}^{b} r^2 + n\sum_{i=1}^{a}\sum_{j=1}^{b} s^2 + \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} t^2 \quad \text{\_\_}(6)$$

That is,

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2 = bn\sum_{i=1}^{a}(\bar{y}_{i..} - \bar{y}_{...})^2 + an\sum_{j=1}^{b}(\bar{y}_{.j.} - \bar{y}_{...})^2$$
$$+ n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 + \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2 \quad \text{\_\_}(7)$$

where,

$$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2 \quad \text{\_\_\_}(8)$$

$$SS_A = bn\sum_{i=1}^{a}(\bar{y}_{i..} - \bar{y}_{...})^2 \quad \text{\_\_\_}(9)$$

$$SS_B = an\sum_{j=1}^{b}(\bar{y}_{.j.} - \bar{y}_{...})^2 \quad \text{\_\_}(10)$$

$$SS_{AB} = n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 \quad \text{\_\_}(11)$$

$$SS_E = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2 \text{ \_\_(12)}$$

That is,

$$SS_T = SS_A + SS_B + SS_{AB} + SS_E \text{\_\_(13)}$$

Alternative computation formulae

From equation __(8)

$$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2$$

From RHS

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk}^2 - 2y_{ijk}\,\bar{y}_{...} + \bar{y}_{...}^2) \text{ \_\_(14)}$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\bar{y}_{...}\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk} + abn\,\bar{y}_{...}^2 \text{ \_\_(15)}$$

But $\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk} = y_{...}$ and $\bar{y}_{...} = \frac{y_{...}}{abn}$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\frac{y_{...}}{abn}y_{...} + abn\left(\frac{y_{...}}{abn}\right)^2 \text{ \_\_(16)}$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\frac{y_{...}^2}{abn} + abn\,\frac{y_{...}^2}{(abn)^2} \text{ \_\_(17)}$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\frac{y_{...}^2}{abn} + \frac{y_{...}^2}{abn} \text{ \_\_(18)}$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn} \text{ \_\_(19)}$$

Therefore

$$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{...})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn} \text{ \_\_(20)}$$

From equation __(9)

$$SS_A = bn\sum_{i=1}^{a}(\bar{y}_{i..} - \bar{y}_{...})^2$$

From RHS

$$bn\sum_{i=1}^{a}(\bar{y}_{i..} - \bar{y}_{...})^2 = bn\sum_{i=1}^{a}(\bar{y}_{i..}^2 - 2\bar{y}_{...}\bar{y}_{i..} + \bar{y}_{...}^2) \text{ \_\_(21)}$$

$$= bn\sum_{i=1}^{a}\bar{y}_{i..}^2 - 2\bar{y}_{...}\,bn\sum_{i=1}^{a}\bar{y}_{i..} + abn\,\bar{y}_{...}^2 \text{ \_\_(22)}$$

$$= bn\sum_{i=1}^{a}\left(\frac{y_{i..}}{bn}\right)^2 - 2\bar{y}_{...}\,bn\sum_{i=1}^{a}\frac{y_{i..}}{bn} + abn\left(\frac{y_{...}}{abn}\right)^2 \text{ \_\_(23)}$$

$$= \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - 2\frac{y_{...}}{abn}\sum_{i=1}^{a}y_{i..} + \frac{y_{...}^2}{abn} \text{ \_\_(24)}$$

But $\sum_{i=1}^{a} y_{i..} = y_{...}$

$$= \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - 2\frac{y_{...}}{abn}y_{...} + \frac{y_{...}^2}{abn} \text{ \_\_(25)}$$

$$= \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - 2\frac{y_{...}^2}{abn} + \frac{y_{...}^2}{abn} \text{ \_\_(26)}$$

$$= \sum_{i=1}^{a} \frac{y_{i..}^2}{bn} - \frac{y_{...}^2}{abn} \_\_(27)$$

Therefore,

$$SS_A = bn\sum_{i=1}^{a}(\bar{y}_{i..} - \bar{y}_{...})^2 = \sum_{i=1}^{a} \frac{y_{i..}^2}{bn} - \frac{y_{...}^2}{abn} \_\_(28)$$

From equation __(10)

$$SS_B = an\sum_{j=1}^{b}(\bar{y}_{.j.} - \bar{y}_{...})^2$$

From RHS

$$an\sum_{j=1}^{b}(\bar{y}_{.j.} - \bar{y}_{...})^2 = an\sum_{j=1}^{b}(\bar{y}_{.j.}^2 - 2\bar{y}_{...}\bar{y}_{.j.} + \bar{y}_{...}^2) \_\_(29)$$

$$= an\sum_{j=1}^{b}\bar{y}_{.j.}^2 - 2\bar{y}_{...}\, an\sum_{j=1}^{b}\bar{y}_{.j.} + abn\,\bar{y}_{...}^2 \_\_(30)$$

$$= an\sum_{j=1}^{b}\left(\frac{y_{.j.}}{an}\right)^2 - 2\bar{y}_{...}\, an\sum_{j=1}^{b}\frac{y_{.j.}}{an} + abn\left(\frac{y_{...}}{abn}\right)^2 \_\_(31)$$

$$= \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - 2\frac{y_{...}}{abn}\sum_{j=1}^{b} y_{.j.} + \frac{y_{...}^2}{abn} \_\_(32)$$

$$\text{But } \sum_{j=1}^{b} y_{.j.} = y_{...}$$

$$= \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - 2\frac{y_{...}}{abn}y_{...} + \frac{y_{...}^2}{abn} \_\_(33)$$

$$= \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - 2\frac{y_{...}^2}{abn} + \frac{y_{...}^2}{abn} \_\_(34)$$

$$= \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - \frac{y_{...}^2}{abn} \_\_(35)$$

Therefore,

$$SS_B = an\sum_{j=1}^{b}(\bar{y}_{.j.} - \bar{y}_{...})^2 = \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - \frac{y_{...}^2}{abn} \_\_(36)$$

From equation __(12)

$$SS_E = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2$$

From RHS

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij.})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk}^2 - 2y_{ijk}\,\bar{y}_{ij.} + \bar{y}_{ij.}^2) \_\_(37)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}\bar{y}_{ij.} + n\sum_{i=1}^{a}\sum_{j=1}^{b}\bar{y}_{ij.}^2 \_\_(38)$$

$$\text{But } \sum_{k=1}^{n} y_{ijk} = y_{ij.}$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\sum_{i=1}^{a}\sum_{j=1}^{b} y_{ij.}\bar{y}_{ij.} + n\sum_{i=1}^{a}\sum_{j=1}^{b}\bar{y}_{ij.}^2 \_\_(39)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\sum_{i=1}^{a}\sum_{j=1}^{b} y_{ij.}\left(\frac{y_{ij.}}{n}\right) + n\sum_{i=1}^{a}\sum_{j=1}^{b}\left(\frac{y_{ij.}}{n}\right)^2 \_\_(40)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - 2\sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} + \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} \quad \_\_(41)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} \quad \_\_(42)$$

Therefore,

$$SS_E = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk}-\bar{y}_{ij.})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} \quad \_\_(43)$$

From equation __(13)

$$SS_T = SS_A + SS_B + SS_{AB} + SS_E$$

That is, $SS_{AB} = SS_T - SS_A - SS_B - SS_E$ ___(44)

$$= \left(\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn}\right) - \left(\sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - \frac{y_{...}^2}{abn}\right) - \left(\sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - \frac{y_{...}^2}{abn}\right) - \left(\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n}\right) \quad \_\_(45)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn} - \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} + \frac{y_{...}^2}{abn} - \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} + \frac{y_{...}^2}{abn} - \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 + \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} \quad \_\_(46)$$

$$= \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} - \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} + \frac{y_{...}^2}{abn} \quad \_\_(47)$$

Therefore,

$$SS_{AB} = n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} - \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} + \frac{y_{...}^2}{abn} \quad \_\_(48)$$

Typical table of a two-factor factorial design with n replicates per cell

		Factor B				Total ($y_{i..}$)	Average ($\bar{y}_{i..}$)
		1	2	...	b		
Factor A	1	y_{111} y_{112} ⋮ y_{11n}	y_{121} y_{122} ⋮ y_{12n}	...	y_{1b1} y_{1b2} ⋮ y_{1bn}	$y_{1..}$	$\bar{y}_{1..}$
	2	y_{211} y_{212} ⋮ y_{21n}	y_{221} y_{222} ⋮ y_{22n}	...	y_{2b1} y_{2b2} ⋮ y_{2bn}	$y_{2..}$	$\bar{y}_{2..}$
	⋮	⋮	⋮	...	⋮	⋮	⋮
	a	y_{a11} y_{a12} ⋮ y_{a1n}	y_{a21} y_{a22} ⋮ y_{a2n}	...	y_{ab1} y_{ab2} ⋮ y_{abn}	$y_{a..}$	$\bar{y}_{a..}$
Total ($y_{.j.}$)		$y_{.1.}$	$y_{.2.}$	………	$y_{.b.}$	$y_{...}$	
Average ($\bar{y}_{.j.}$)		$\bar{y}_{.1.}$	$\bar{y}_{.2.}$	………..	$\bar{y}_{.b.}$		$\bar{y}_{...}$

ANOVA table for two-factor factorial design with n replicates per cell

Source of variation	Sum of squares	Degrees of freedom	Mean Square	F_{ratio}
Factor A	SS_A	$a-1$	$MS_A = \frac{SS_A}{(a-1)}$	$F_A = \frac{MS_A}{MS_E}$
Factor B	SS_B	$b-1$	$MS_B = \frac{SS_B}{(b-1)}$	$F_B = \frac{MS_B}{MS_E}$
Interaction (AB)	SS_{AB}	$(a-1)(b-1)$	$MS_{AB} = \frac{SS_{AB}}{(a-1)(b-1)}$	$F_{AB} = \frac{MS_{AB}}{MS_E}$
Error	SS_E	$ab(n-1)$	$MS_E = \frac{SS_E}{ab(n-1)}$	
Total	SS_T	$abn-1$		

The F_{ratio} is calculated by dividing each of the mean squares by the mean squares error to obtain the corresponding F_{ratio}. The hypotheses tests were carried out at α (5%) significance level and the decision rule was to reject the null hypothesis (H_0) if the calculated *Sig.* value (*p*-value) is less than the α (5%).

Test concerning difference between paired samples

The paired sample *t*-test, sometimes called the dependent sample *t*-test, is a statistical procedure used to determine whether the mean difference between two sets of observations is zero. In a paired sample *t*-test, each subject or entity is measured twice, resulting in *pairs* of observations. Common applications of the paired sample *t*-test include case-control studies or repeated-measures designs.

The test procedure, called the (matched paired *t*-test) is appropriate when:

1. Each sample is drawn from a normal or near-normal population.
2. The test is conducted on paired data (as a result, the data sets are not independent).
3. The standard deviation of the population's difference is unknown.

The test procedure is as follows:

1. To test H_0: $\mu_d = 0$ vs H_1 : $\mu_d \neq 0$
   Use a two-tailed test and reject H_0 if $t_{cal} \geq t_{tab:\frac{\alpha}{2},n-1}$ or $t_{cal} \leq -t_{tab:\frac{\alpha}{2},n-1}$
2. To test H_0: $\mu_d = 0$ vs H_1 : $\mu_d > 0$
   Use a one-tailed test and reject H_0if $t_{cal} \geq t_{tab:\alpha,n-1}$
3. To test H_0: $\mu_d = 0$ vs H_1 : $\mu_d < 0$
   Use a one tailed test and reject H_0 if $t_{cal} \leq -t_{tab:\alpha,n-1}$

Where $\mathrm{d_i} = \mathrm{X_{1i}} - \mathrm{X_{2i}}$ (Difference between the population samples).

$\mathrm{X_{1i}} \in$ Group 1 and $\mathrm{X_{2i}} \in$ Group 2.

The test statistic is:

$$t_{cal} = \frac{\bar{d}}{s_{\bar{d}}} \quad \text{\_\_(49)}$$

where

$$\bar{d} = \frac{\sum_{i=1}^{n} d_i}{n} \quad \text{\_\_(50)}$$

$$S_{\bar{d}} = \frac{\mathrm{S_d}}{\sqrt{\mathrm{n}}} \quad \text{\_\_(51)}$$

$$\mathrm{S_d} = \sqrt{\frac{\sum(d-\bar{d})^2}{n-1}} = \sqrt{\frac{\sum d^2 - n\bar{d}^2}{n-1}} \quad \text{\_\_(52)}$$

Multiple regression analysis

The multiple linear regression model is an extension of a simple linear regression model to incorporate two or more explanatory variable in a prediction equation for a response variable. Multiple regression modelling is now a mainstay of statistical analysis in most fields because of its power and flexibility (Tabachnick and Fidell, 1996).

Multiple regression examines how two or more variables act together to affect the dependent variable. This allows researchers to introduce control variables that may account for observed relationships, as well as document cumulative effects (Tabachnick and Fidell, 1996).

While the interpretation of the statistics in multiple regression is, on the whole, the same as in bivariate regression, there is one important difference. In bivariate regression, the regression coefficient is interpreted as the predicted change in the value of the dependent variable for a one-unit change in the independent variable. In multiple regression, the effects of multiple independent variables often overlap in their association with the dependent variable. This means a variable's coefficient shows the "net strength" of the relationship of that particular independent variable to the dependent variable, above and beyond the relationships of the other independent variables. Each coefficient is then interpreted as the predicted change in the value of the dependent variable for a one-unit change in the independent variable, after accounting for the effects of the other variables in the model (Tabachnick and Fidell, 1996).

Naturally, if we add more factors to our model that are useful for explaining *y*, then more of the variation in *y* can be explained. Thus, multiple regression analysis can be used to build better models for predicting the dependent variable.

The general multiple linear regression model can be written in the population as:

$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k + \mu_i$

y_i is the dependent variable

$x_1, x_2, \ldots, x_k$ are independent variables

β_o is the intercept

$\beta_1, \beta_2, \ldots, \beta_k$ are regressor coefficients

μ_i is the error term

No matter how many explanatory variables we include in our model, there will always be factors we cannot include, and these are collectively contained in μ_i.

VIII. Research Methodology

Research design

The design adopted for this study is experimental design. In this study, a total of 30 male albino rats weighing 160-180 grams (11 weeks old) were used. We then constructed cages with aluminium nets and woods. The cages were placed in rat house, Moshood Abiola Polytechnic. The initial weight of each rat was taken both for drug preparation and comparison after experiment before being placed in the cage. They were all fed with basal feed and water only for one week before the commencement of the experiment in order for their body system to adapt to the new environment.

Thereafter, using the lottery method, allocation of the rats to cages was done on paper and then carried out on the field to ensure random allocation of the rats and the cages to prevent experimental error. The rats were divide into 5 groups where 4 groups received Paracetamol (Standard), Ibucap (Caffeine Ibuprofen and Paracetamol), Cenpain (Diphenhydramine Hcl and Paracetamol), and *Orphesic* (Orphenadrine Citrate and Paracetamol) respectively three times daily for 10 days. The 5th group (control group) received basal feed and water only.

We ensured that they were housed under standard conditions with access to food and water, and acclimatized to laboratory conditions for one week before the commencement of the experiments.

All experiments were carried out according to Helsinki principles on care and use of animals. The data collected was modelled as Two-factor factorial experiment with 3 replicates per cell and analysed electronically using SPSS 21 (IBM version) and Minitab 17.

The models under consideration are:

Model I: $y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk}$

y_{ijk} = Sperm count

μ = Grand mean

A_i = Effect of varieties of paracetamol combinations

B_j = Effect of different dosage of varieties of paracetamol combinations

$(AB)_{ij}$ = Interaction effect between A and B.

Where $i = 1, 2, \ldots, 5$; $j = 1, 2$; $k = 1, 2, 3$

Model II: $y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6 + \beta_7 x_7 + \beta_{13} x_{13} + \beta_{14} x_{14} + \beta_{15} x_{15} + \beta_{16} x_{16} + \beta_{17} x_{17} + \beta_{23} x_{23} + \beta_{24} x_{24} + \beta_{25} x_{25} + \beta_{26} x_{26} + \beta_{27} x_{27} + \mu_i$

y_i = Sperm count

x_1 and x_2 = Different dosage of varieties of paracetamol combinations

x_3 to x_7 = Varieties of paracetamol combinations

x_{13} to x_{27} = Interaction between different dosage of varieties of paracetamol combinations and varieties of paracetamol combinations.

Experimental and data collection method

Varieties of paracetamol combinations

Paracetamol (standard)

Ibucap: Caffeine Ibuprofen and Paracetamol

Cenpain: Diphenhydramine Hcl and Paracetamol

Orphesic: Orphenadrine Citrate and Paracetamol

Haematological Study

30 Adult male albino rats were randomly divided into 10 groups with each group consisting of 3 rats. The 10 groups of rats were subjected to the following oral treatments thrice a day for 10 days.

Group 1 rats received Paracetamol only (500g)
Group 2 rats received Ibucap (500g)
Group 3 rats received Cenpain night (500g)
Group 4 rats received Orphesic (500g)
Group 5 rats received Basal feed and water only
Group 6 rats received Paracetamol only (1000g)
Group 7 rats received Ibucap (1000g)
Group 8 rats received Cenpain night (1000g)
Group 9 rats received Orphesic (1000g)
Group 10 rats received Basal feed and water only.

Drug preparation

Ten UC (Universal bottles) bottles were prepared, each labelled according to the number of cages, and the quantity of drugs required were placed into each UC bottle according to the weight calculations.

The content of each UC bottle was then pressed using a mortar and pestle to allow quick dissolving of the drugs in distilled water. Then we measured 9ml and 14.4ml of distilled water and inserted it into each UC bottle containing 500 and 1000 milligram of varieties of paracetamol respectively to prepare the drug solution and shake properly. The solution was used for two days, after which another preparation was made using the same method.

About twenty-four hours after the 10th day of drugs administration, the animals were then euthanized by cervical dislocation for semen extraction.

Semen extraction

Materials used in semen extraction are:

(i) Microscope (ii) Blade (iii) Microscope slides (iv) Coverslip

(v) Eosin nigrostain (vi) Normal selin (vii) Surgical scissors.

Sperm collection

The testes were removed along with the epididymis, the claudal epididymis were separated from the testes, blotted with filter paper and lacerated to collect semen.

Motility

This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (27^{0C}) and two drops of warm sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope.

Life/dead ratio

This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air; the stained slide was immediately examined under the microscope.

The live sperm cells were unstained while the dead sperm cells absorbed the stain, the stained and unstained sperm were counted and the percentage was calculated.

Sperm morphology

This was done by adding two drops of warm Walls and Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and air-dried; the stained slide was immediately examined under the microscope.

Sperm count

This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5ml formol-saline in a graduated test-tube and the volume of fluid displaced was taken as the volume of the epididymis. It is then taken to the lab since the machine cannot be moved.

Testicular history

After weighing the testes, they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70% alcohol. The tissues were then cut in slabs of about 0.5cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70% alcohol for 2 hours, 95% alcohol for 2 hours, 100% alcohol for 2 hours, 100% alcohol for 2 hours and finally 100% alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene.

Thereafter, the tissues were infiltrated in molten Paraffin wax for 2 hours in an oven at 57^0C, after which the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5μm).

The satisfactory ribbons were picked up from a water bath (50-55^0C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it.

The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1% acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70%, 90% and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute.

Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at x40, x100 and x400 magnifications.

After the data was collected, it was statistically analysed electronically for appropriate inferences using SPSS 21(IBM version) and Minitab 17.

Method of data analysis

Data for this research was collected primary via experimental method. Collected data was analysed by partitioning the design model into:

$$SS_{Total} = \sum_{i=1}^{5}\sum_{j=1}^{2}\sum_{k=1}^{3} {y_{ijk}}^2 - \frac{y_{\dots}^2}{30}$$

With 29 degrees of freedom

$$SS_{\substack{Varieties\ of \\ paracetamol}} = \sum_{i=1}^{5} \frac{{y_{i..}}^2}{6} - \frac{y_{\dots}^2}{30}$$

With 4 degrees of freedom

$$SS_{\substack{Different\ dosage\ of \\ varieties\ of\ paracetamol}} = \sum_{j=1}^{2} \frac{{y_{.j.}}^2}{15} - \frac{y_{\dots}^2}{30}$$

With 1 degree of freedom

$$SS_{Interaction} = \sum_{i=1}^{5}\sum_{j=1}^{2} \frac{{y_{ij.}}^2}{3} - \frac{y_{\dots}^2}{30} - SSA - SSB$$

With 4 degrees of freedom

$$SS_{Error} = SS_{Total} - SS_{\substack{Varieties\ of \\ paracetamol}} - SS_{\substack{Different\ dosage\ of \\ varieties\ of\ paracetamol}} - SS_{Interaction}$$

With 20 degrees of freedom

Data presentation

Table 1: Sperm Count (in millions)

Dosage	*Varieties of paracetamol*				
	Paracetamol	**Ibucap**	**Cenpain**	**Orphensic**	**Control**
500mg	9.8	7.2	8.9	6.8	13.3
	11.8	7.8	7.1	7.6	14.8
	10.1	8.1	6.9	8.1	14.1
1000mg	9.8	9.8	11.2	6.9	14.9
	8.5	8.8	10.2	8.0	14.8
	8.9	8.9	11.8	8.2	14.9

Table 2: Motility (in percentage)

Dosage	*Varieties of paracetamol*				
	Paracetamol	**Ibucap**	**Cenpain**	**Orphensic**	**Control**
500mg	80	60	70	80	95
	80	60	60	60	95
	80	60	70	60	90
1000mg	70	70	70	60	95
	70	70	70	60	98
	70	70	80	60	97

Table 3: Life/Death Ratio (in percentage)

Dosage	*Varieties of paracetamol*				
	Paracetamol	**Ibucap**	**Cenpain**	**Orphensic**	**Control**
500mg	98	85	98	85	98
	95	95	85	95	98
	98	98	85	98	98
1000mg	95	98	95	95	98
	98	95	98	98	95
	95	98	98	85	97

Table 4: Volme of Sperm (in millions)

Dosage	*Varieties of paracetamol*				
	Paracetamol	**Ibucap**	**Cenpain**	**Orphensic**	**Control**
500mg	5.2	5.2	5.1	5.2	5.2
	5.1	5.2	5.2	5.1	5.2
	5.2	5.3	5.3	5.2	5.2
1000mg	5.2	5.2	5.1	5.2	5.2
	5.1	5.1	5.2	5.2	5.2
	5.2	5.2	5.2	5.2	5.2

Table 5: Weight (gram)

S/N	Initial (Before drug administration)	Final (After drug administration)
1	150	120
2	120	160
3	160	160
4	190	150
5	180	160
6	180	180
7	120	160
8	140	120
9	100	100
10	190	120
11	160	160
12	150	100
13	160	160
14	160	140
15	160	160
16	150	160
17	150	100
18	130	160
19	180	160
20	170	160
21	160	180
22	150	120
23	170	140
24	150	160
25	150	200
26	160	140
27	160	180
28	170	160
29	160	180
30	150	200

- **Data analysis**

Table 6: Descriptive statistics of sperm count

Dosage	Varieties of paracetamol	Mean	Std. Deviation	N
500mg	Standard paracetamol	10.567	1.0786	3
	Ibucap	7.700	.4583	3
	Cenpain	7.633	1.1015	3
	Orphensic	7.500	.6557	3
	Control	14.067	.7506	3
	Total	9.493	2.7426	15
1000mg	Standard paracetamol	9.067	.6658	3
	Ibucap	9.167	.5508	3
	Cenpain	11.067	.8083	3
	Orphensic	7.700	.7000	3

	Control	14.867	.0577	3
	Total	10.373	2.6285	15
Total	Standard paracetamol	9.817	1.1479	6
	Ibucap	8.433	.9223	6
	Cenpain	9.350	2.0695	6
	Orphensic	7.600	.6164	6
	Control	14.467	.6470	6
	Total	9.933	2.6771	30

Dependent Variable: Sperm count

Table 7: Levene's test of equality of error variances
(Homogeneity of variance or Homoscedasticity)

F	df1	df2	Sig.
1.660	9	20	.165

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a. Design: Dosage + VP + Dosage * VP
Dependent Variable: Sperm count

Table 8: Tests of between-subjects effects (ANOVA)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Model	3157.033[a]	10	315.703	576.803	.000
Dosage	5.808	1	5.808	10.611	.004
VP	171.597	4	42.899	78.379	.000
Dosage * VP	19.495	4	4.874	8.905	.000
Error	10.947	20	.547		
Total	3167.980	30			
a. R Squared = .997 (Adjusted R Squared = .995)					

Dependent Variable: Sperm count

Table 9: Tukey's multiple comparison (Post Hoc) tests for varieties of paracetamol

(I) Varieties of paracetamol	(J) Varieties of paracetamol	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Standard P.	Ibucap	1.383*	.4271	.030	.105	2.661
	Cenpain	.467	.4271	.808	-.811	1.745
	Orphensic	2.217*	.4271	.000	.939	3.495
	Control	-4.650*	.4271	.000	-5.928	-3.372
Ibucap	Standard P.	-1.383*	.4271	.030	-2.661	-.105
	Cenpain	-.917	.4271	.240	-2.195	.361
	Orphensic	.833	.4271	.324	-.445	2.111
	Control	-6.033*	.4271	.000	-7.311	-4.755
Cenpain	Standard P.	-.467	.4271	.808	-1.745	.811
	Ibucap	.917	.4271	.240	-.361	2.195
	Orphensic	1.750*	.4271	.005	.472	3.028
	Control	-5.117*	.4271	.000	-6.395	-3.839
Orphensic	Standard P.	-2.217*	.4271	.000	-3.495	-.939
	Ibucap	-.833	.4271	.324	-2.111	.445
	Cenpain	-1.750*	.4271	.005	-3.028	-.472
	Control	-6.867*	.4271	.000	-8.145	-5.589
Control	Standard P.	4.650*	.4271	.000	3.372	5.928
	Ibucap	6.033*	.4271	.000	4.755	7.311
	Cenpain	5.117*	.4271	.000	3.839	6.395
	Orphensic	6.867*	.4271	.000	5.589	8.145

Based on observed means. The error term is Mean Square (Error) = .547.
*. The mean difference is significant at the .05 level.
Dependent Variable: Sperm count

Table 10: Tukey's homogeneous subset for sperm count

Varieties of paracetamol	N	Subset			
		1	2	3	4
Orphensic	6	7.600			
Ibucap	6	8.433	8.433		
Cenpain	6		9.350	9.350	
Standard P.	6			9.817	

Control	6				14.467
Sig.		.324	.240	.808	1.000

Means for groups in homogeneous subsets are displayed. Based on observed means
The error term is Mean Square (Error) = .547.
a. Uses Harmonic Mean Sample Size = 6.000.
b. b. Alpha = .05.

Table 11: Estimated marginal means for dosage

Dosage	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
500mg	9.493	.191	9.095	9.892
1000mg	10.373	.191	9.975	10.772

Dependent Variable: Sperm count

Table 12: Estimated marginal means for varieties of paracetamol

Varieties of paracetamol	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Standard paracetamol	9.817	.302	9.187	10.447
Ibucap	8.433	.302	7.803	9.063
Cenpain	9.350	.302	8.720	9.980
Orphensic	7.600	.302	6.970	8.230
Control	14.467	.302	13.837	15.097

Dependent Variable: Sperm count

Table 13: Estimated marginal means for interaction between dosage and varieties of paracetamol levels

Dosage	Varieties of paracetamol	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
500mg	Standard paracetamol	10.567	.427	9.676	11.458
	Ibucap	7.700	.427	6.809	8.591
	Cenpain	7.633	.427	6.742	8.524
	Orphensic	7.500	.427	6.609	8.391
	Control	14.067	.427	13.176	14.958
1000mg	Standard paracetamol	9.067	.427	8.176	9.958
	Ibucap	9.167	.427	8.276	10.058
	Cenpain	11.067	.427	10.176	11.958
	Orphensic	7.700	.427	6.809	8.591
	Control	14.867	.427	13.976	15.758

Dependent Variable: Sperm count

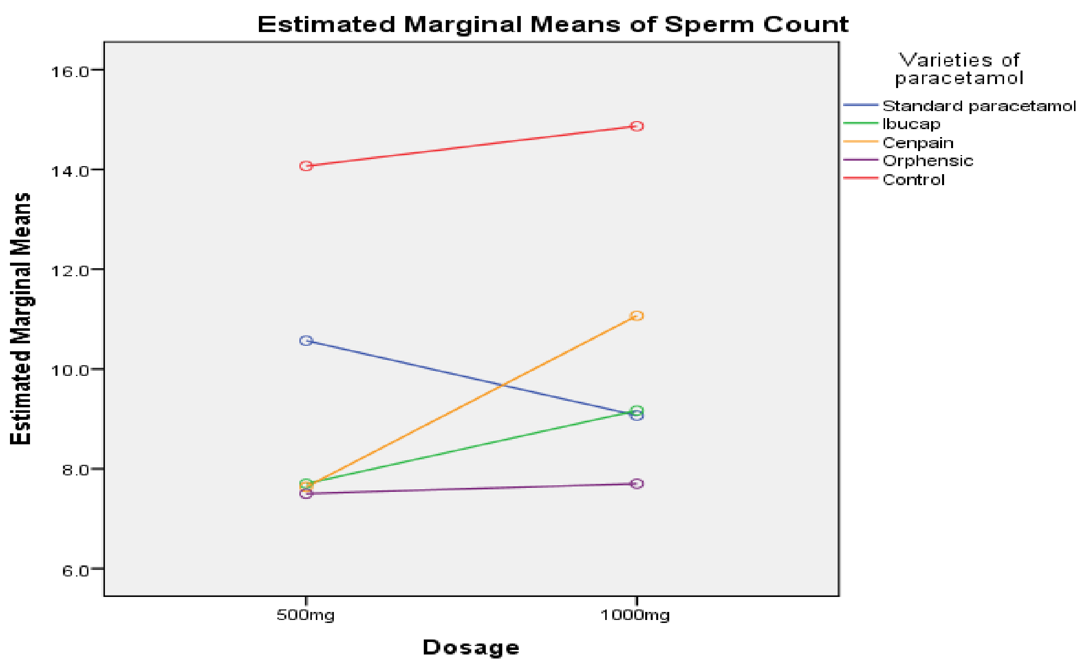

Fig.1: Profile plot for dosage and varieties of paracetamol

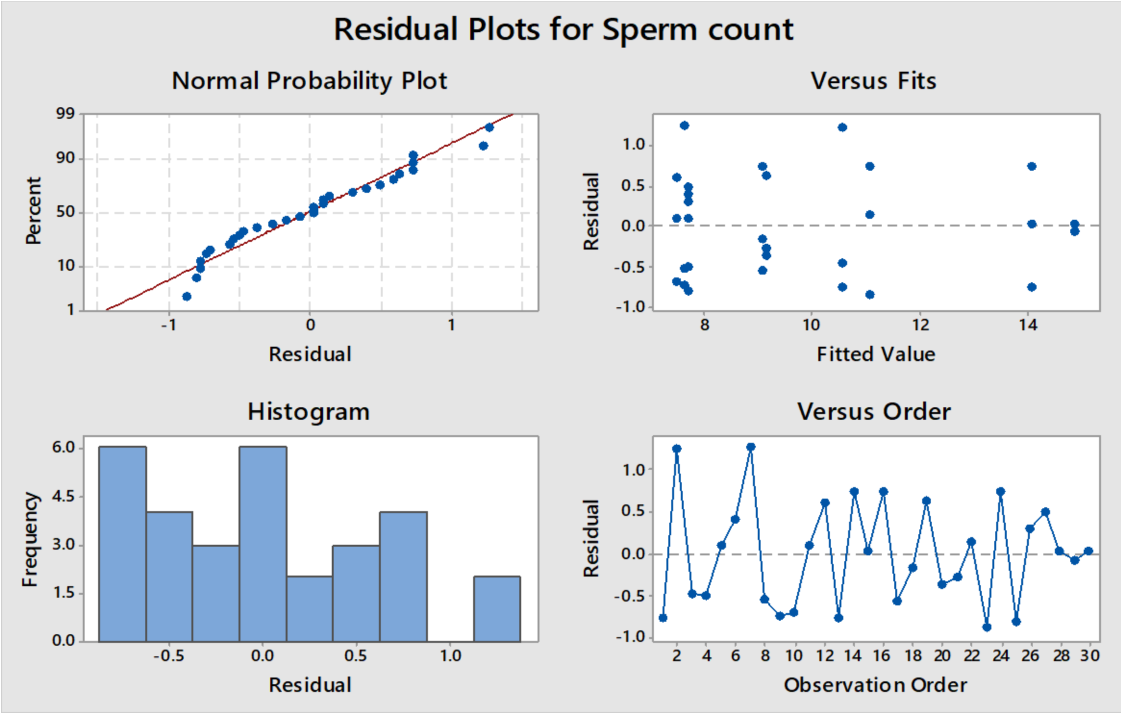

Fig.2: Residual plots for sperm count

Table 14: Regression coefficients

Term	Coef	SE Coef	T-Value	*p*-value	VIF
Constant	9.933	0.135	73.54	0.000	
Dosage					
500mg	-0.440	0.135	-3.26	0.004	1.00
1000mg	0.440	0.135	3.26	0.004	*
Varieties					
Standard paracetamol	-0.117	0.270	-0.43	0.670	1.60
Ibucap	-1.500	0.270	-5.55	0.000	1.60
Cenpain	-0.583	0.270	-2.16	0.043	1.60
Orphensic	-2.333	0.270	-8.64	0.000	1.60
Control	4.533	0.270	16.78	0.000	*
Dosage*Varieties					
500mg Standard P.	1.190	0.270	4.41	0.000	1.60
500mg Ibucap	-0.293	0.270	-1.09	0.290	1.60
500mg Cenpain	-1.277	0.270	-4.73	0.000	1.60
500mg Orphensic	0.340	0.270	1.26	0.223	1.60
500mg Control	0.040	0.270	0.15	0.884	*
1000mg Standard P.	-1.190	0.270	-4.41	0.000	*
1000mg Ibucap	0.293	0.270	1.09	0.290	*
1000mg Cenpain	1.277	0.270	4.73	0.000	*
1000mg Orphensic	-0.340	0.270	-1.26	0.223	*
1000mg Control	-0.040	0.270	-0.15	0.884	*

Regression Equation

Sperm count = 9.933 - 0.440 Dosage_1 + 0.440 Dosage_2 - 0.117 Varieties of paracetamol_1
- 1.500 Varieties of paracetamol_2 - 0.583 Varieties of paracetamol_3
- 2.333 Varieties of paracetamol_4
+ 4.533 Varieties of paracetamol_5
+ 1.190 Dosage*Varieties of paracetamol_1 1
- 0.293 Dosage*Varieties of paracetamol_1 2
- 1.277 Dosage*Varieties of paracetamol_1 3
+ 0.340 Dosage*Varieties of paracetamol_1 4
+ 0.040 Dosage*Varieties of paracetamol_1 5
- 1.190 Dosage*Varieties of paracetamol_2 1
+ 0.293 Dosage*Varieties of paracetamol_2 2
+ 1.277 Dosage*Varieties of paracetamol_2 3
- 0.340 Dosage*Varieties of paracetamol_2 4
- 0.040 Dosage*Varieties of paracetamol_2 5

Table 15: Model Summary

S	Rq	R-sq(adj)	R-sq(pred)
0.739820	94.73%	92.36%	88.15%

Table 16: Paired t-test for Initial - After weight of rats

	N	Mean	StDev	SE Mean
Initial	30	156.00	20.10	3.67
After	30	151.67	27.05	4.94
Difference	30	4.33	30.14	5.50

95% lower bound for mean difference: -5.02
T-Test of mean difference = 0 (vs > 0): T-Value = 0.79 P-Value = 0.219

IX. Results and discussion

Summary of results

	Test of Homogeneity of variance or Homoscedasticity	
	Sig.	*Remark*
Levene's test	.165	Population variances are equal.

	Tests of between-subjects effects (ANOVA)	
	Sig.	*Remark*
Dosage	0.004	Significant difference in the effect of different dosage of varieties of paracetamol combinations on sperm count
Varieties of Paracetamol (VP)	0.000	Significant difference in the effect of varieties of paracetamol combinations on sperm count
Dosage * VP	0.000	Significant interaction effect between dosage and varieties of paracetamol on sperm count

	Tukey's multiple comparison (Post Hoc) test for varieties of paracetamol				
	Standard paracetamol	Ibucap	Cenpain	Orphensic	Control
Standard paracetamol		0.030*	0.808	0.000*	0.000*
Ibucap			0.240	0.324	0.000*
Cenpain				0.005*	0.000*
Orphensic					0.000*
Control					

	Regression coefficients	
	p-value	Effect remark
Dosage		
500mg	0.004	Significant effect on sperm count
1000mg	0.004	Significant effect on sperm count
Varieties		
Standard P.	0.670	Insignificant effect on sperm count
Ibucap	0.000	Significant effect on sperm count
Cenpain	0.043	Significant effect on sperm count
Orphensic	0.000	Significant effect on sperm count
Control	0.000	Significant effect on sperm count
*Dosage*Varieties*		
500mg Standard P.	0.000	Significant effect on sperm count
500mg Ibucap	0.290	Insignificant effect on sperm count
500mg Cenpain	0.000	Significant effect on sperm count
500mg Orphensic	0.223	Insignificant effect on sperm count
500mg Control	0.884	Insignificant effect on sperm count
1000mg Standard P.	0.000	Significant effect on sperm count
1000mg Ibucap	0.290	Insignificant effect on sperm count
1000mg Cenpain	0.000	Significant effect on sperm count
1000mg Orphensic	0.223	Insignificant effect on sperm count
1000mg Control	0.884	Insignificant effect on sperm count

	Paired t-test			
	Mean	*Mean Difference*	*p-value*	*Remark*
Initial Weight	156.00	4.33	0.219	No significant difference in the mean weights of rat before and after drugs administration.
Final Weight	151.67			

*. The mean difference is significant at the .05 level.

Discussion of results

The descriptive statistics table (Table 6) shows that rats administered 500mg of Standard paracetamol with basal feed approximately produce an average of 10.567 million sperm count. Those administered 500mg of Ibucap with basal feed approximately produce an average of 7.700 million sperm count, those administered 500mg of Cenpain with basal feed approximately produce an average of 7.633 million sperm count, those administered 500mg of Orphensic with basal feed approximately produce an average of 7.500 million sperm count and those fed with basal feed alone approximately produce an average of 14.067 million sperm count.

Rats administered 1000mg of Standard paracetamol with basal feed approximately produce an average of 9.067 million sperm count, those administered 1000mg of Ibucap with basal feed approximately produce an average of 9.167 million sperm count, those administered 1000mg of Cenpain with basal feed approximately produce an average of 11.067 million sperm count, those administered 1000mg of Orphensic with basal feed approximately produce an average of 7.700 million sperm count and those fed with basal feed alone approximately produce an average of 14.867 million sperm count.

The Levene's test table (Table 7), used before comparison of means, assess the equality of variances for a variable calculated for two or more groups. The assumption is that variances of the populations from which different samples are drawn are equal. It test the null hypothesis that the population variances are equal (called homogeneity of variance or homoscedasticity). This table gives a *Sig.* value of 0.165, which is greater than the conventional level of significance ($\alpha = 0.05$) and implies that the null hypothesis of equal variances is accepted. In other words, there is no difference between the variances in the population. Hence the validity of the use of analysis of variance (ANOVA).

The ANOVA table (Table 8) gives a *Sig.* value of 0.000 for varieties of paracetamol combinations, which is less than the conventional level of significance ($\alpha = 0.05$) implies that the null hypothesis of equal effect is rejected. In other words, there is significant difference in the effect of varieties of paracetamol combinations on sperm count. A *Sig.* value of 0.004 for different dosage of varieties of paracetamol combinations, which is less than the conventional level of significance ($\alpha = 0.05$) implies that the null hypothesis of equal effect is rejected. In other words, there is significant difference in the effect of different dosage of varieties of paracetamol combinations on sperm count. A *Sig.* value of 0.000 for interaction between varieties of paracetamol combinations and different dosage, which is less than the conventional level of significance ($\alpha = 0.05$) implies that the null hypothesis of ineffective interaction is rejected. In other words, there is significant interaction effect between varieties of paracetamol combinations and different dosage on sperm count.

Since there is significant difference in the effect of varieties of paracetamol combinations on sperm count, there is need to carryout multiple comparison test. The Post Hoc tests (Table 9) shows that there is significant difference in the effect of Standard paracetamol and Ibucap, in the effect of Standard paracetamol and Orphensic, in the effect of Standard paracetamol and Control, in the effect of Ibucap and Control, in the effect of Cenpain and Orphensic, in the effect of Cenpain and Control, and in the effect of Orphensic and Control.

The Tukey's homogeneous subset (Table 10) shows that Orphensic is significantly different from Cenpain, Standard paracetamol and Control because it never appears in any subset with Cenpain, Standard paracetamol or Control. Ibucap is significantly different from Standard paracetamol and Control because it never appears in any subset with either Standard paracetamol or Control. Cenpain is significantly different from Orphensic and Control because it never appears in any subset with either Orphensic or Control. Standard paracetamol is significantly different from Orphensic, Ibucap and Control because it never appears in any subset with Orphensic, Ibucap or Control. Control is significantly different from all other groups as it does not appear in a subset together with any of the groups.

The regression model for the sperm count (y_i) is deduced as:

$$\begin{aligned} y_i = 9.933 - 0.440x_1 + 0.440x_2 - 0.117x_3 - 1.500x_4 \\ - 0.583x_5 - 2.333x_6 + 4.533x_7 \\ + 1.190x_{13} - 0.293x_{14} - 1.277x_{15} \\ + 0.340x_{16} + 0.040x_{17} - 1.190x_{23} \\ + 0.293x_{24} + 1.277x_{25} - 0.340x_{26} \\ - 0.040x_{27} \end{aligned}$$

The regression coefficient (Table 14) shows that the two dosages (500mg and 1000mg) of varieties of paracetamol combinations under study individually have significant effects on sperm count. Of the four varieties of paracetamol combinations under study and control, only the standard paracetamol individually does not have a significant effect on sperm count (with a *p*-value of 0.670). In addition, of the ten interactions, significant interaction effects on sperm count is found in 500mg of Standard paracetamol, 500mg of Cenpain, 1000mg of Standard paracetamol and 1000mg of Cenpain.

The model summary table (Table 15) gives the coefficient of determination (R^2) value as 94.73%. This implies that approximately 94.73% of the variation in sperm count is being explained by varieties of paracetamol combinations, different dosage of varieties of paracetamol combinations and the interactions.

The paired t-test (Table 16) for rats' weights gives a *p*-value of 0.219, which is greater than the conventional level of significance ($\alpha = 0.05$). This implies that there is no significant difference in the mean weights of rat before and after drugs administration. In other words, long term paracetamol usage does not reduce body weight.

X. Conclusions and recommendations

Conclusions

From the analysis it can be concluded that:

1. Rats fed with basal feed only but controlled at 1000mg dosage approximately produce the largest sperm count of 14.867 million on the average, while those administered 500mg of Orphensic with basal feed approximately produce the least sperm count of 7.500 million on the average.
2. The varieties of paracetamol combinations significantly have different effects on sperm count. Similarly, dosages of varieties of paracetamol combinations also significantly have different effects on sperm count. In addition, there is significant interaction effect between varieties of paracetamol combinations and different dosage on sperm count.
3. The two dosages (500mg and 1000mg) of varieties of paracetamol combinations individually have significant effects on sperm count.
4. Of the four varieties of paracetamol combinations and

control, only the standard paracetamol individually does not have a significant effect on sperm count.
5. Of the ten interactions, significant interaction effects on sperm count is found in 500mg of Standard paracetamol, 500mg of Cenpain, 1000mg of Standard paracetamol and 1000mg of Cenpain.
6. Varieties of paracetamol combinations, different dosage of varieties of paracetamol combinations and the interaction under study contribute approximately 94.73% to sperm count.
7. Long term paracetamol usage does not significantly reduce body weight.

Recommendations

Our study showed that paracetamol or acetaminophen as an analgesic and antipyretic drug may affect sperm count positively or negatively, significantly or insignificantly. It should be noted that these effects are dose dependent and are seen both in short and long-terms of drug consumption. We advise that men especially should be careful in acetaminophen usage without the prescription of their physician. They should be conscious of the level of consumption of parcetamol. Most specifically, cautious should be taken when taking 500mg of Standard paracetamol, 500mg of Cenpain, 1000mg of Standard paracetamol and 1000mg of Cenpain.

References

1. Abarikwu S. O. (2013). *Causes and risk factors for male-factor infertility in Nigeria: A review*. Afr J. Reprod Health. 2013 Dec; 17(4): 150-166.
2. Aksu E. H., Ozkaraca M., Kandemir F. M., Omur A.D., Eldutar E., Kucukler S. and Comakli S. (2016). *Mitigation of paracetamol-induced reproductive damage by chrysin in male rats via reducing oxidative stress*. First International Journal of Andrology. Andrologia 2016, 28, 1145-1154.
3. Amy D. J. (2009). *Small Animal Critical Care Medicine*. Pages 334-337. Chapter79: Acetaminophen
4. Banihani S. A. (2017). *Effect of paracetamol on semen quality*. First International Journal of Andrology. Andrologia 2017.
5. Clive R. I. (2010). *Experimental Statistics for Agriculture and Horticulture*. CABI Pulishing.
6. David M. K. (2018). *Ibuprofen linked to male infertility: Study by Nick McDermott*. Extracted from https://nypost.com/2018/01/08/ibuprofen-linked-to-male-infertility-study/
7. David W. R. (2017). Effects of paracetamol on adults. University of Texas Southwestern Medical Center, Dallas.
8. Ibrahim T., Agnihotri S., Agnihotri A. K. (2013) *Paracetamol Toxicity - An Overview*. Emergency Med 3:158. doi:10.4172/2165-7548.1000158
9. Kennon-Mcgill S. and Mcgill M. R. (2018). *Extrahepatic toxicity of acetaminophen: critical evaluation of the evidence and proposed mechanisms*. J Clin Transl Res. 2018 Jan 15; 3(3). pii: 5. doi: 10.18053/jctres.03.201703.005.
10. Oyedeji K. O., Bolarinwa A. F. and Ojeniran S. S. (2013). *Effect of paracetamol (acetaminophen) on haematological and reproductive parameters in male albino rats*. Journal of Pharmacy and Biological Sciences. Volume 4, Issue 6, (Jan. – Feb. 2013), pp 65-70.
11. Ratnasooriya W. D. and Jayakody A. (2001). *Long-term administration of large doses of paracetamol impairs the reproductive competence of male rats*. Asian Journal of Andrology. 2(4): 247-255. January 2001.
12. Rodriguez L. A. G. and Hernandez-Diaz S. (2001). *The risk of upper gastrointestinal complications associated with nonsteroidal anti-inflammatory drugs, glucocorticoids, acetaminophen, and combinations of these agents*. Arthritis Research. February 2001. 3(2): 98-101. Available online http://arthritis-research.com/content/3/2/098
13. Samarawickrama A. A. H. S., Suraweera R.K., Sivayoganathan C., Sakeena M.H.F. (2014). *A study on Paracetamol consumption by undergraduate students in the Faculty of Allied Health Sciences, University of Peradeniya*. International Journal of Scientific and Research Publications. Volume 4, Issues 6, June 2014. pp. 1-6
14. Suneil A. and Babak K. (2018). *Acetaminophen Toxicity*. StatPearls Publishing. Available online https://www.ncbi.nlm.nih.gov/books/NBK441917/
15. Tabachnick B. G. and Fidell L. S. (1996). Using Multivariate Statistics (3rd edition). New York: Harper Collins.
16. World Health Organization (1999, 2010). World Health Organization reference values for human semen characteristics

Online

https://www.statisticssolutions.com/manova-analysis-paired-sample-t-test/
https://www.theturekclinic.com/blog/